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Heredity

FOUNDATIONS OF MODERN BIOLOGY SERIES

Heredity

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PRENTICE-HALL FOUNDATIONS OF MODERN BIOLOGY SERIES

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Foundations of Modern *Biology* Series

The science of biology today is *not* the same science of fifty twenty-five, or even ten years ago Today's accelerated pace of research, aided *by new instruments techniques, and points* of view imparts to biology a rapidly changing character as discoveries pile one on top of the other All of us are aware, however that each new and important discovery is not just a mere addition to our knowledge-it also throws our established beliefs into question, and forces us constantly to reappraise and often to reshape the foundations upon which biology rests. An adequate presentation of the dynamic state of modern biology is, therefore, a formidable task and a challenge worthy of our best teachers.

The authors of this series believe that a new approach to the organization of the subject matter of biology is urgently needed to meet this challenge, an approach that introduces the student to biology as a growing, active science, and that also *permits each teacher of biology to determine the level and the structure of his own course.* A single textbook cannot provide such flexibility and it is the author's strong conviction that these student needs and teacher prerogatives can best be met by a series of short, inexpensive, well-written, and well-illustrated books so planned as to encompass those areas of study central to an understanding of the content, state, and direction of modern biology The FOUNDATIONS OF MODERN BIOLOGY SERIES represents the translation of these ideas into print, with each volume being complete in itself yet at the same time serving as integral part of the series as a whole.

Preface

"For after all, my dear sir the chicken may merely be an egg's way of making another egg. In this book, the author wishes to pay tribute and offer his gratitude to the mechanism chosen by the egg to replenish its kind, for this mechanism has much to do with heredity. The study of heredity is in its infancy but it has already established itself as a science in its own right—the science of genetics. Within this field, we can and do formulate hypotheses, make predictions, and subject them to rigorous test—quantitative experiments. Young it is, but genetics has already attracted the attention of many giants and is the first of the biological sciences to enjoy the concerted efforts of biologists, chemists, and physicists.

Like all sciences, genetics has many facets. It deals with the practical problems of foods and drugs, of intelligence and behavior and the explosive problems of population. In fact, genetics intrudes in all walks of life. Its investigators have diverse interests and aspirations, yet through them all runs a common thread, an intense curiosity about genetic material, its nature, its potentialities, and its manner of transmission. In the field of heredity there are many points of view for the science is busy on many fronts. My colleagues may well cry in anguish at the biased views in this presentation. But so brief a book on the subject makes arbitrary decisions inevitable, and here we elect to discuss one aspect that binds geneticists of all persuasions together—the gene and what it does.

While the discussion of heredity in this volume is centered around the gene,

genetics impinges on all aspects of biology—on physiology biochemistry growth and development, structure and function, to name only a few These are fields covered by the other books of this series, and we urge that they be read, for armed with more extensive knowledge of modern biology you will be able to enrich your understanding of the many sided field of heredity

Writing, like heredity requires genes The author's genetic endowment in writing is modest, but luckily he enjoys generous and able colleagues. In the planning and writing of this book, the collaboration of these colleagues, especially of Dr Stanley E. Mills, and the editorial assistance of James M Gulher Jr was invaluable and the author is indebted to them for whatever clarity sophistication, and polish the volume possesses.

David M Bonner

Contents

Heredity

The Material Basis of Heredity

The word heredity is simply the name given to the means by which living organisms reproduce their kind. An understanding of heredity, therefore, requires knowledge of the various processes involved in reproduction. In recent years, this knowledge has increased dramatically. The combined efforts of an ever increasing number of research scientists, and the development of new tools and experimental techniques of great power have already revealed much, and promise more. Many questions that have puzzled generations of biologists have been answered. More important, however, new information has bred new questions, provoked a desire for deeper levels of comprehension. Today, with the descriptive elements of reproduction largely clarified and behind us, we are going after the very atoms of heredity. We want to know about their arrangements, their architecture and their activities, the chemical transformations and the physical forces that provide for the continuation of life.

We can begin by noting a fact obvious to all of us. Living systems are highly complex. For example, the most elementary studies soon reveal the wealth of biochemical activities that a cell must contain just to survive. Yet we also know from everyday observation that each organism must have not only the means to maintain itself, but information for reproducing systems like itself as well. The best evidence available today indicates that this information is contained

of substances which is passed on from one generation to the next, and which in large measure prescribes the nature of the succeeding generation. These substances we refer to as "the material basis of heredity

The scientific study of the material basis of heredity began in the latter part of the nineteenth century with the work of the Austrian monk, Gregor Mendel. His experimental results led him to the formulation of the "laws of heredity which, in turn, provided the inspiration for the research that continues at an accelerated pace today Of great current interest is the fact that recent work in this field has brought about a revolution in modern experimental biology It has led to the development of what is now known as molecular biology in which the disciplines of biology chemistry and physics have fused to create a field of challenge and excitement. It is this field that shows explosive experimental activity at the present time and it is the author s bias that molecular biology offers the best opportunity for gaining insight into the basic problems of biology as they are now defined Hence the present discussion of heredity will be centered around the developments of recent years, developments using as experimental tools microorganisms rather than higher plants or higher animals. Our hope is that in this way it will be possible not only to present clearly the principles underlying the science of heredity i.e., genetics but at the same time to give some insight into the problems of the present and the fascination of the future.

The Cellular Elements

All organisms consist of cells. Moreover whether they consist of one cell or of thousands, they grow and reproduce by cell division. A bacterium is a single cell. It grows until it has doubled in size then reproduces by splitting in two. The result of this division two identical cells from one. Man is a multicellular organism and he, too grows by increase in cell size and division of his constituent cells. He reproduces by producing certain cells (gametes) sperm (male) and eggs (female) which fuse to give rise to a single fertilized egg cell (zygote) The zygote is capable of growing and dividing, and of ultimately giving rise to a new multicellular individual. The information needed to reproduce a complex multicellular individual is thus transmitted through a single cell, and each dividing cell in turn must contain the information required to reproduce itself The elements of heredity belong to the cell, therefore rather than to the organism, and, in considering the material basis of heredity we are free to focus on the processes involved in the reproduction of single cells.

What elements within c ll can be expected to carry hereditary information? Consider the structur of a cell. The basic structures of most cells are remarkably similar (Fig. 1) A cell is surrounded by a cell membrane and sometimes an outer cell wall. Within the membrane there is the nucleus and cytoplasm The nucleus of most cells is a discrete body hav

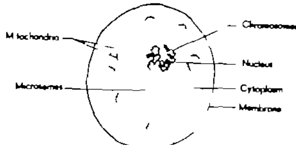


Fig. 1 Diagrammatic representation of cell.

ing a nuclear membrane and containing thread like structures (chromosomes) The cytoplasm contains a number of discrete elements. Mitochondria (small particles having a defined and characteristic morphology) are found in profusion in the cytoplasm, and, by means of an electron microscope, we can see still smaller particles (microsomes) Thus the cytoplasm is found to contain different kinds of particulate elements, varying in number and, as will be seen later in function, while in general but a single nucleus is found per cell.

The cellular elements that carry hereditary information must be present in every reproducing cell In addition, to account for the fact that like faithfully begets like, these elements must be capable of reproducing or dividing accurately The nucleus fulfills these requirements. For example, every cell capable of reproduction has a nucleus- cells lacking one cannot reproduce. Not every cell has a well-defined nucleus, the bacteria, for example, but even they have characteristic nuclear bodies. During cell division, the nucleus divides by an elaborate mechanism (mitosis) that leads to the production of an exact copy of each constituent chromosome. It is the chromosomes in the nucleus that carry the material basis of heredity

Chromosomes had been seen in nuclei for a great many years before their function was known. The proof that chromosomes are hereditary elements came from the work of Thomas Hunt Morgan and his colleagues working with the fruit fly Drosophila. To understand how it could be shown that chromosomes are the hereditary elements, we must consider briefly the experiments of Gregor Mendel. Mendel, working with the common garden pea, found that certain characteristics or traits appeared to be inherited independently of one another For instance he mated a strain that formed tall plants and had wrinkled seed coats to one that formed short plants and had smooth seed coats. In the progeny the trait for tallness (as against shortness) was transmitted in equal numbers to the plants that had wrinkled seed coats and to those with smooth seed coats. These observations led Mendel to conclude that in sexual reproduction the inheritance of one trait is independent of the inheritance of another

Many years later however Morgan and his associates, studying the fruit fly found that certain combinations of traits were transmitted to-

gether suggesting that the hereditary elements determining these traits were, in some way linked. Analysis of the inheritance of many traits showed that they could be separated into several groups. Within each group, the traits, and therefore the hereditary elements controlling them, were inherited together i.e., they were linked. On the other hand, the inheritance of traits, each of which fell into a different group, showed the independence observed by Mendel. In *Drosophila melanogaster* four and only four linkage groups could be found. Cytological observations revealed that the cells of Drosophila contain precisely four distinct pairs of chromosomes. An interesting coincidence.

Morgan thereupon concluded that the chromosomes must be the hereditary elements, and he was right. This can be proven by cytological study of the giant chromosomes present in the cells of the salivary glands in Drosophila. For example a fly was found to have an abnormal trait, and this was reflected in an abnormality in the structure of one of its chromosomes. When this fly was mated to a normal fly and the progeny examined, it developed that every fly with the abnormal chromosome had the abnormal trait. All the normal progeny had the normal chromosome. Thus the combined use of cytology and genetic analysis led to the clear proof that the chromosomes of the nucleus carry the genetic material.

The number of chromosomes per nucleus is characteristic and constant for each living organism. The somatic cells of higher plants and animals contain duplicate sets of chromosomes (diploid=2N) while the gametic cells contain a single set (haploid=N) The gametic cells of Drosophila have a chromosome number of 4, the somatic cells have 8. The haploid number for human cells is 23, for the fungus *Neurospora crassa* 7 and for the bacterium *E. coli* 1. There is a clear species specificity with respect to chromosome number and chromosome morphology

Mitosis

Since chromosomes carry the hereditary information, we would expect them to be accurately reproduced and transmitted in an orderly and precise way They are. Sometime during the life history of the cell, an exact copy of each chromosome is synthesized. When cell division is triggered, a sequence of events occurs which results in the formation of two cells with identical sets of chromosomes. This type of nuclear division is called mitosis. At first glance, the various stages of mitosis appear overwhelmingly complicated, but what is accomplished is simple. In brief the chromosomes contract and gather independently of one another on a plane approximately in the center of the cell. Each chromosome is then seen to be tightly paired with its previously synthesized copy The sister strands, or chromatids, then separate, one to each end of the cell, and two identical sets of chromosomes are formed. The nuclei reorganize, and the cell divides in two A mitotic cell division, then, is simply a division that



Fig. 2. Photomicrograph of mitosis in the whitefish. When cell division is triggered, the displaced chromosomes gather and appear as distinct bodies the nucleus. The sister chromosomes are separated, and two new cells are formed. (Copyright by General Biological Supply House, Inc., Chicago.)

produces two cells, each with an identical nucleus. This is shown in Fig. 2.

Meiosis

Both haploid and diploid cells can undergo mitosis; in fact, mitosis is characteristic of nearly all cells. Diploid cells, however, can undergo an additional type of nuclear division, meiosis, which results in the reduction in chromosome number from diploid to haploid. Such a division is obviously necessary when gametic cells are formed from somatic cells. In man, for instance, a new generation results from the fusion of a sperm cell and an egg cell. The gametic cells were both formed by individuals having 46 chromosomes and, upon fusion, must give rise to a new indi-

DNA The Genetic Material

Since both cytological and genetic evidence give overwhelming proof that chromosomes carry the hereditary material, a study of the chemical nature of chromosomes might be expected to give insight into the chemical basis of heredity. Within recent years, it has been possible to isolate chromosomes from the other constituents of the cell and thereby carry out chemical analysis of chromosomes. It turns out that chromosomes are complex, not only in terms of morphology but in chemical terms as well. They do not consist of a single chemical substance, but are composed, chiefly of three types of substances: proteins, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA).

A brief word about these three substances. They are naturally occurring polymers. A polymer is a large molecule formed from a few simple molecules (the mers) repeatedly linked in chemical bondage. Proteins are made up of various combinations of twenty simple molecules—amino acids—and different proteins may have anywhere from 100 to 10 000 units. DNA and RNA are generally made by repeated linkage of four simple units called nucleotides. A DNA molecule may have as many as 30,000 units strung together. The chemical difference between DNA and RNA, the significance of the order in which the units are linked (primary structure) and the total number of each unit per molecule (composition) will be discussed later. It is sufficient for now to note that from pure chemical analysis of chromosomes, we are

not able to conclude whether one of these three substances contains the genetic information, or whether it is contained in some combination. This is perhaps not too surprising. Proof of this point must come ultimately from biological tests.

In order to establish that a certain substance contains genetic information, we would like to isolate the substance in pure form from one organism and demonstrate that when it is put into another organism, traits of the first organism appear *in the second and are passed on to the progeny* of the second—a simple and sensitive test. To date, it has not been possible to do this with material obtained from chromosomes of higher plants or *animals, but it has been possible to do precisely this with certain bacteria.*

Transformation

Curiously enough, the lines of inquiry that led to an understanding of the chemical nature of genetic material arose from a study of the pestilent organism, *Diplococcus pneumoniae* which causes pneumonia. During the 1920's, two bacteriologists carried out a series of experiments in which they showed that certain strains of the pneumococcus bacterium, when inoculated into animals, would not cause symptoms of the disease. Such strains are called avirulent. If animals were inoculated with a virulent strain, the animals contracted pneumonia (Fig. 5) If animals were inoculated with cells of a virulent strain that previously had been killed by treatment with heat, the animals showed no symptoms. However when animals were inoculated with both avirulent and heat killed virulent cells, neither alone sufficient for disease production, symptoms of pneumonia *did appear and, upon isolation of the organism giving rise to these symptoms, a virulent strain was found. Moreover the strain retained its viru* lence through countless cell divisions. The presence of heat-killed virulent cells, therefore, had the curious effect of changing avirulent cells to virulent cells, which is a change in a genetic trait. This phenomenon was called bacterial transformation, and its study by Oswald Avery and his collaborators at the Rockefeller Institute opened the way to the identification of the chemical nature of the genetic material.

It is now known that many inherited bacterial traits will undergo transformation, and that it can be carried out in a test tube as well as in mice. For instance, again with the pneumococcus organism as an example, certain strains are killed by the antibiotic, streptomycin (and are thus called streptomycin-sensitive) This sensitivity is passed on from one cell to another at cell division. Other strains are known to be resistant to streptomycin, also a heritable trait. Suppose we perform the following experiment (see Fig. 6) Grow up a large mass of streptomycin-resistant bacteria. Collect the bacteria, grind them up until all the cells are shattered, then make a dilute salt extract of the mass and discard the insoluble cell debris. Add some extract of the resistant-strain to a growing population

Fig. 5. Bacterial transformation in living animals (in vivo)

of a sensitive strain. Many cells of the sensitive strain are now converted to streptomycin-resistant cells, and they pass this resistance on to their progeny. Extracts of streptomycin-resistant cells, therefore, have the effect of causing a permanent hereditary change from streptomycin sensitivity to streptomycin resistance.

It then proved possible, by chemical fractionation, to isolate the substance in such extracts that causes this change in genetic traits. It was shown that the DNA present in such extracts is the active component.

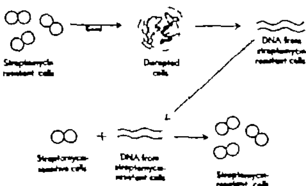


Fig. 6. Bacterial transformation in the test tube (in vitro) using purified DNA and the trait streptomycin resistance

DNA can be isolated from streptomycin-resistant cells and highly purified. When this purified DNA is added to the medium in which streptomycin-sensitive cells are growing, transformation of streptomycin-sensitive cells to streptomycin-resistant cells will occur. This extraordinary phenomenon, molecular sex if you will, may not captivate you from an aesthetic point of view but its importance in terms of its contribution to the understanding of genetic material cannot be overestimated.

Transformation can be demonstrated in other bacteria. Certain strains of a fat rod called *Bacillus subtilis* can grow in a medium that does not contain the amino acid, tryptophan. Tryptophan is necessary for life. It is one of the essential units of nearly all proteins, and life as we know it cannot exist without proteins. The bacterium usually obtains its required supply of tryptophan by synthesizing it from other molecules. This is called a tryptophan-independent strain. There are other strains of *Bacillus subtilis* that cannot make tryptophan and must have it supplied in their environment if they are to grow and divide—tryptophan-dependent strains. *Both properties, tryptophan dependence and tryptophan independence,* are heritable.

DNA, extracted and purified from a tryptophan-independent strain, when added to a growing culture of a dependent strain, will transform many of the cells to tryptophan independence.

DNA thus fulfills the criteria set forth earlier i.e., for a certain chemical substance to be designated as containing the genetic information of a given organism, it should be present in the nucleus or nuclear bodies and we should be able to isolate the substance and demonstrate that when it is put into another organism, traits of the donating organism appear in the second. Neither RNA nor protein are active in bacterial transformation. Thus we may conclude that DNA is the chemical substance which serves as the basis of heredity

Bacterial Viruses

A second line of inquiry that has also led to identification of the genetic material as DNA is the study of bacterial viruses. For a better understanding of the nature of the evidence, it would be well to describe a typical bacterium and bacterial virus.

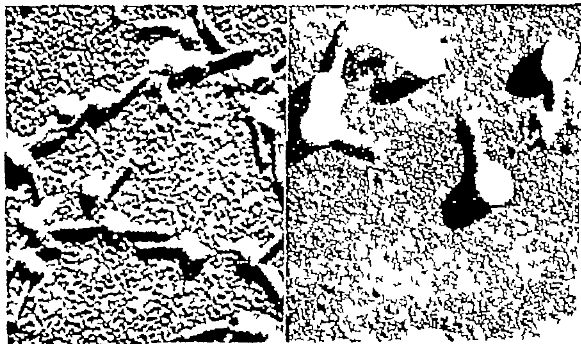
Bacteria are microscopic, single-celled organisms. They have characteristic nuclear like bodies (nucleoids) which contain DNA. Bacterial cells also contain large amounts of RNA, among other substances. Bacterial viruses are still smaller but they can be seen by means of an electron microscope (Fig. 8) Bacterial viruses cannot reproduce in the absence of living bacterial cells, and they are found to consist of only protein and DNA.

The life cycle of a typical bacterial virus (bacteriophage or just phage) is given in Fig. 9 The life cycle may be divided into three distinct

Fig. 7 Photomicrogr ph the cells of B cereus shew t show uclea bodies. (Courtesy Dr C F Robinow.)

phases the infective phase, the vegetative phase and the progeny formation phase The infective particle which was shown in Fig. 8, attaches to the bacterial cell wall by its tail. A break in the cell wall is induced, and the contents of the infecting particle are then injected into the bacterial cell. This step is of great interest, because the injecting par

Fig. 8. Electron micrographs of two different bacterial viruses. (Courtesy Dr L W Labaw.)



ticle has a protein coat around a core consisting mainly of DNA. Thus the material that is injected into the bacterial cell and that initiates the formation of new virus particles is DNA.

After injection of the virus DNA, the bacterial cell goes into a period of vegetative virus formation during which new virus DNA and protein are synthesized inside the bacterium. In the final stage of this twenty-minute life cycle, the newly synthesized virus protein and DNA are assembled into new virus particles, the cell ruptures, and the viruses spill out into the medium. *The point of particular interest here* is the fact that the material which is injected into the bacterial cell, and which initiates the formation of new virus particles, is DNA. The genetic information of bacterial viruses is therefore contained in DNA. Study of both bacterial transformation and bacterial viruses consequently leads to the conclusion that DNA is the chemical basis of heredity.

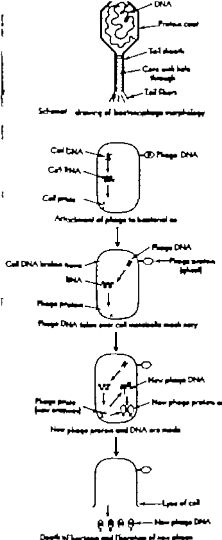


Fig. 9 The life cycle of typical bacterial virus or bacteriophage. The infective phase consists of attachment and injection. The vegetative phase is the period required for the formation of new phage protein and DNA. Progeny formation requires the assembly of phage DNA and protein into new infective particles.

Infective RNA

This conclusion, however, brings up a contemporary problem of great importance. Many different viruses are, of course, known. They are all extraordinarily small in size, and they all have an obligate requirement for a living host for replication. In addition to bacterial viruses, plant and animal viruses are well known. While bacterial viruses are found to consist of protein and DNA, many plant and animal viruses are known that consist of protein and RNA instead. Tobacco mosaic virus, a well-known virus of tobacco plants, is of this latter type, as is the virus that causes

human poliomyelitis. Using the tobacco mosaic virus (TMV) we can carry out some very interesting experiments. By chemical means, it is possible to separate the protein and RNA components of TMV (These can be put back together in the test tube, where some stable viruses are reformed thus at least some of the protein and RNA survive the separation uninjured.) If we inoculate a tobacco plant with just the protein fraction, nothing happens. If, however just the RNA component is inoculated, new TMV particles are produced that have both the protein and the RNA components. The TMV RNA, therefore, carries all the information necessary for the synthesis of both the TMV protein and the RNA.

In view of such observations, how sure are we that, in higher organisms, the genetic information is coded in the DNA of the chromosomes? While infective RNA is known in plant and animal viruses, we still know relatively little about the manner in which it replicates. Recent experimental evidence suggests that its replication involves an interaction with the DNA of the host cell. The fact that DNA is the only substance which, if transferred from one *cell* to a second *cell* results in hereditary changes in the second *cell* (transformation) is a strong indication that it is the primary substance in which genetic information is coded. The *translation* of genetic information into action involves, as we will see, RNA. The two compounds are intimately related, and the very closeness of this relationship could well result in RNA exhibiting an apparent primary genetic function under certain circumstances, as in TMV

At the present time, therefore, it appears reasonable to conclude that in organisms, in general, the genetic information is coded in the DNA of the chromosomes, and that the RNA is involved in the translation of the information into action. For the moment, we need not concern ourselves with the chemical structure of these compounds, for their structure will be of greater interest after we consider how DNA transmits hereditary information and how it can determine the characteristics of specific cells. Armed with the knowledge that the material basis of heredity is DNA, let us next consider how this substance acts.

Genes and Biochemical Reactions

Long before the discovery of DNA as the chemical basis of heredity the inheritance of well-defined characteristics in insects and domestic plants had been studied by means of the basic tool of genetics, the inheritance test. The inheritance test consists of controlled matings and careful observation of the resulting progeny For example, two plants which differ in a given trait such as color are mated (crossed) and the distribution of color in their offspring, and the descendants of their offspring, is then analyzed. This test is as old as genetics, and was the experimental tool that enabled Mendel to formulate his laws of inheritance.

Detailed study of the transmission of specific traits reveals that they appear to be determined by specific areas of a chromosome. The specific area of a chromosome that determines a specific trait *will be termed a gene.* *Genes* exist in alternative forms (if this were not true, the inheritance test would be impossible) The alternative forms of a single gene are called alleles. For example, one aspect of the property of blood-clotting in man is determined by a specific region in one of his chromosomes. The possession of this region in one allelic form insures normal blood-clotting after injury On the other hand, a carrier of an alternative form of this very same chromosomal region suffers from a disease known as hemophilia in which blood-clotting occurs very slowly if at all. Certain genes are [illegible] to have as many as 100 different [illegible] and as we become more [illegible]

paying, we will probably find that all genes exist in many alternate forms.

How then, are the genes assembled? Through patient and careful study of the transmission of specific traits, individually and in combination, a concept of the genetic structure of a chromosome emerged that can be likened to beads on a string. Each chromosome can be represented as a single string of beads, with each bead representing a unit of genetic material, the gene. The alleles of a given gene would correspond to different forms or colors of a particular bead. A particular bead is present on only one string at a time, and, whatever the form or color of the bead (allele) it is always in the same place on the string.

Neurospora Life Cycle

To clarify and extend these ideas we will consider in some detail inheritance in the fungus, *Neurospora crassa*. Neurospora was chosen because it has attracted the attention of a group of outstanding scientists whose brilliant experiments with this organism have contributed much to our current understanding of the biochemical basis of gene action.

Neurospora normally grows as a spreading mycelium. The mycelium is composed of individual strands, hyphae, which contain many nuclei in a common cytoplasm. All the nuclei have one set of chromosomes, i.e., they are haploid. (See Fig. 10) Vegetative or asexual reproduction occurs by growth of the hyphae and repeated nuclear division. Asexual reproduction also occurs by formation of asexual spores called conidia. Note

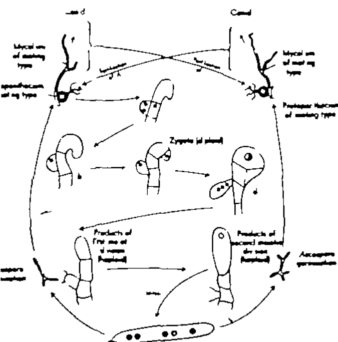


Fig. 10. The life cycle of Neurospora.

particularly that the mature fungus is haploid (as opposed to, say man who is diploid) and that asexual reproduction is based entirely on mitotic divisions of haploid nuclei. Neurospora also undergoes sexual reproduction. Sexual reproduction requires the fusion of two haploid nuclei to give rise to a diploid nucleus which then undergoes a reduction division by meiosis. Fusion of the two haploid nuclei occurs through the union of two strains of opposite mating types. These strains are indistinguishable, morphologically but they can be shown to differ in mating type by the fact that if strains of opposite mating type are grown together characteristic sexual spores (ascospores) are formed. Strains of similar mating type, if grown together do not form such spores.

The two mating types are designated *A* and *a*. In sexual reproduction, then, a cell will be formed containing one haploid nucleus from strain *A* and one haploid nucleus from strain *a*. The nuclei fuse and give a diploid cell. The fusion nucleus then undergoes meiosis in a sac-like structure called an *ascus*. Let's go over this carefully and give it your close attention. You will find it rewarding in understanding what follows.

Each nucleus brings to the sexual fusion an identical set of chromosomes. Consider any given pair of chromosomes. In the fusion nucleus of the ascus, they show strong mutual attraction and come to lie very close to one another each gene on one chromosome closely paired with its allele on the other chromosome. (The forces responsible for this pairing are as yet unknown.) At this time, you will recall, each member of the pair appears as if it had split in two. As previously described, what is now essentially 4 chromosomes (a tetrad) undergo 2 reduction divisions and form 4 haploid nuclei, the products of meiosis Each tetrad distributes itself independently of any other tetrad, and haploid nuclei are produced that can contain some chromosomes from one parent and some from the other

Each of the 4 products of meiosis then divides mitotically to give 2 identical nuclei, and the final result is 8 nuclei that stem from the original diploid nucleus. Spore walls are formed around each nucleus to form 8 ascospores in the ascus. Each pair of spores represents a product of meiosis, and the individual ascospores may be germinated to give rise to a new haploid, vegetative organism. An interesting aspect of meiosis in Neurospora resides in the sac-like structure, the ascus. The ascus is narrow enough so that in the divisions following the first division of meiosis, the resultant nuclei cannot slip past one another The mature ascus thus contains 8 spores in linear array and the order of the ascospores reflects the order of their formation during meiosis.

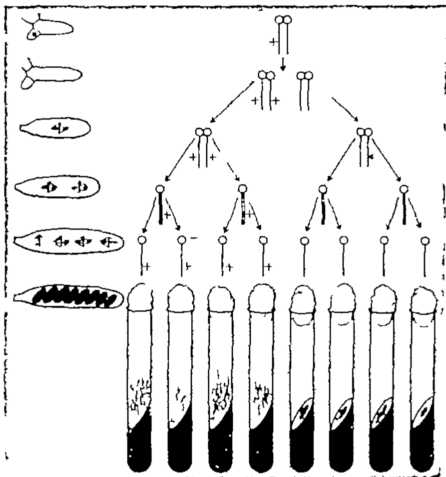
The Inheritance Test in Neurospora

Let us now consider the transmission of a specific trait in this organism. Neurospora normally grows as a spreading mycelium. A variant form

is known which shows a button like or colonial type of growth. The colonial type can be mated with a normal strain and their progeny examined. Upon dissection and germination of the eight ascospores resulting from such a cross, four will show the colonial growth of the variant and four the normal spreading growth. Half of the progeny are like one parent, the other half like the second. This would be expected if the trait—spreading growth versus colonial growth—is determined by a single gene. The normal strain has a gene (+) on one chromosome that determines spreading growth. The colonial strain has an alternative form of this same gene (c) that results in colonial growth.

As shown in Fig. 11, the fusion nucleus formed by the mating of these two strains contains two homologous chromosomes, one bearing the

Fig. 11 The inheritance of a single-gene difference. Gene (c) determines colonial-type growth while its normal allele determines spreading growth. The sequence at the left indicates the nuclear behavior. Note that segregation occurs at the first division, giving a 4:4 spore arrangement in the ascus. (After Beadle.)



normal gene (+) and the second the colonial allele (*c*) At meiosis, these two homologous chromosomes pair and form a tetrad. At the first division, the replicated members of the pair are drawn to opposite ends of the ascus, and so segregate the (+) and (*c*) alleles from each other The second division s spindles are then formed and segregate the sister strands, giving two nuclei having the (+) gene and two nuclei having the (*c*) gene. Each nucleus now undergoes a mitotic division to give four nuclei having the (+) gene and four nuclei having the (*c*) gene. This example clearly illustrates the important general principle that any trait in a haploid organism, such as Neurospora, which is controlled by a *single gene* must show a one-to-one segregation in the progeny This inevitably follows from the fact that there is equal genetic contribution by each parent in the formation of the zygote, and that each homologous chromosome shows equal replication.

Random Assortment

The haploid nucleus of Neurospora contains seven chromosomes. Specific genes are known to be carried on each of the seven chromosomes. This poses the question of how two genes, each carried on a separate chromosome, segregate during meiosis. We can answer the question by studying the transmission of two sets of traits, the traits being determined by genes on different chromosomes. For instance assume that the gene controlling spreading (+) versus colonial (*c*) growth is known to be carried on chromosome No. 1 In addition, assume that a gene on chromosome No. 2 determines the formation of orange conidia (+) while an allele of this gene determines the formation of white or albino conidia (*alb*) Let us analyze the progeny that arise by crossing an orange colonial strain (+ *c*) by an albino spreading strain (*alb* +)

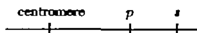
By examining large numbers of spores isolated at random from many asci, we find the following distribution 2ɔ per cent of the progeny are albino and spreading (*alb* +) 25 per cent are orange, colonial (+ *c*) 25 per cent are normal (+ +) and 25 per cent are albino colonial (*alb* *c*) However if we examine the individual spores from single asci, we find that *half* the *asci* examined contained four albino spreading (*alb* +) and four orange colonials (+ *c*) while the other half show four normals (+ +) and four albino colonials (*alb* *c*) Let us consider this phenomenon in terms of the behavior of chromosomes.

The diploid cell or zygote formed by mating a colonial and an albino strain will contain fourteen chromosomes consisting of seven pairs. Chromosome 1 contributed by the colonial strain will carry the gene (*c*) Chromosome 1 contributed by the albino strain will carry the normal allele (+) of the same gene. Chromosome 2 contributed by the albino parent will carry the albino gene (*alb*) while chromosome 2 contributed by the colonial strain will carry the normal allele (+) At meiosis, homologous chromosomes pair and then segregate

At the first meiotic division, chromosome 1 of the albino strain has an equal chance of segregating with its own chromosome 2 or with the chromosome 2 of the colonial strain. If chromosome 1 of the albino strain segregates with its own chromosome 2, an ascus will be formed containing four albino (alb $+$) and four colonial ($+$ c) nuclei. If, however chromosome 1 of the albino strain segregates with chromosome 2 of the colonial strain, recombinant nuclei will be formed. Half the nuclei will carry both the normal genes of chromosomes 1 and 2, and half will carry both the (c) and (alb) genes. This example illustrates a major principle of genetics, namely that the assortment of chromosomes during meiosis is random. Random assortment, in turn, results in genetic recombination.

Linked Genes

Since many genes are carried on a single chromosome, we must also consider the transmission of two genes both of which are carried on the same chromosome. Such genes show the phenomenon of linkage, i.e., they are transmitted together more frequently than would be predicted by chance. Such genes do, however undergo recombination as a consequence of crossing over Consider two gene pairs, $p/+$ and $+/s$ both of which are carried on the same chromosome, with s farther from the centromere than p



The centromere is the point on the chromosome where the sister strands remain attached prior to separation, and it is also the point where the fibers from the spindle attach and draw the strands to opposite poles.

If the strains (p $+$) and ($+$ s) are mated, and progeny ascospores isolated, the majority of the spores upon germination would be of one or the other of the two parental types, either (p $+$) or ($+$ s) However recombinant strains are also found, i e., strains that are normal with respect to both traits ($+$ $+$) as well as the double mutant carrying both the s and p traits (p s) The two *recombinant* classes are invariably present in progeny in equal proportion. The total *number* of recombinant progeny however in this case, is less than that predicted on a basis of random or independent transmission. If these two genes were transmitted independently of each other 25 per cent of the progeny would be like one parent (p, $+$) 25 per cent like the second ($+$ s) 25 per cent would be normal ($+$ $+$) a recombinant class, and 25 per cent would be the double mutant (p, s) the second recombinant class.

The hallmark of linkage is the fact that the total number in the two recombinant classes is significantly less than 50 per cent of the progeny As shown in Fig. 12, recombination of linked genes results from an exchange of segments between non-sister strands in the closely paired tetrad

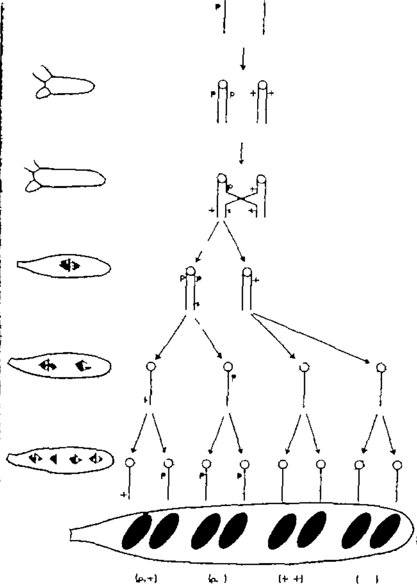


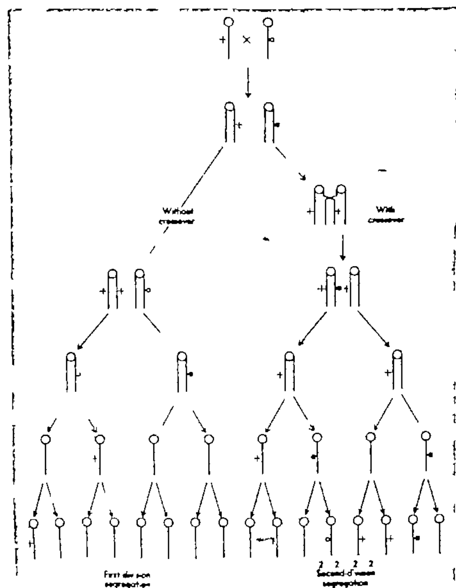
Fig. 12. Exchang between chromatids. Note that segregation occurs after the second division, resulting in 2 2 2 2 spore arrangement in the ascus.

formed in the zygote by homologous chromosomes. The exchange, termed crossing over occurs before the first reduction division. As can be seen in the figure, if a break occurs between genes *p* and *s*, exchange of the terminal segments will result in one strand carrying the two normal alleles (+ +) The other homologous strand will carry the two mutant alleles

(s, p) Note carefully from the figure how the two meiotic divisions then segregate the four different genotypes.

Whether or not a crossover between linked genes has occurred can be readily determined in Neurospora by noting the spore arrangement in the ascus. If no crossover occurs between two genes, the first meiotic division will result in their separation, the ascus will show a 4 4 arrangement, and the two classes will be identical to the parents (see Fig. 11) If, how

Fig. 13. A schematic diagram of the genetic events concerned with first-division segregation and with second-division segregation.



ever a crossover has occurred, segregation will not occur until the second division, it will result in a 2.2 2.2 segregation, and the two parental and two recombinant classes will be present. This is diagrammed in Fig. 13. In the photograph (Fig. 14A) one can clearly see both crossover and noncrossover asci.

Fig. 14. (Top) Asci produced by crossing normal strain of *Neurospora* with strain that shows delayed maturation. Note both first- and second-division segregations. (Courtesy Dr David R. Stadler.) (Bottom) A schematic representation of the gene map of chromosome of *Neurospora crassa*. The numbers after gene indicate different genes affecting the formation of the same end product. The relative distances on the map are derived from recombination data as discussed in the text.

The farther away a gene is from the centromere, then, the greater the chance of a crossover between the gene and centromere, and the greater the chance of a 2 2 2 2 (second-division) segregation. The frequency of first and second-division segregation thus permits the ordering (mapping) of genes relative to the centromere, for the gene closest to the centromere will have the smallest ratio of 1st/2nd division segregation, while the gene farthest away will have the highest. The fact that the chance of a crossover in any given part of a chromosome is about the same for any other part of the chromosome means that the farther apart two genes are, the greater the probability that a crossover can occur The greater the probability that a crossover will occur the higher the frequency of recombination. Thus the frequency of recombination is a measure of the relative distance between two genes. This fact permits the preparation of genetic maps. A map of one chromosome of Neurospora is given in Fig. 14B For a discussion of the details of the construction of such a map consult Chapter 9 of the text by Srb and Owen.[1]

Mutation

Gene configuration is not fixed. As has been mentioned, a given gene can exist in several different forms (alleles) A change of a gene from one form to another is called mutation. Mutation is of paramount importance to the study of heredity for it must be re-emphasized that we can be *aware of the existence of a gene only when more than one allele of that* gene is observed. The rare mutation that occurs spontaneously in nature provides variation and permits the evolution of new and novel forms. Mutation frequency however can be increased experimentally This was first shown by H. J Muller using X rays X-rays and other ionizing radiations increase mutation frequency as do many other agents, e.g., heat, ultraviolet light, and chemicals such as nitrous acid and nitrogen mustard (the mustard gas used in World War I) These and other mutagenic agents are extremely useful in genetic research, since they permit us to explore the possibilities for variation that are inherent in genetic material and to utilize many mutant genes as tools in experiments.

Genes and Nutrition

We are now in a position to consider a problem of primary contemporary interest and research, the mechanism of gene action. The genetic traits we have discussed up to now have been mainly morphological, but we know that a biochemical basis must underlie all such phenomena. Cells of a colonial form must differ from cells of a spreading form in biochemical characteristics, even though the nature of such differences

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1 A.M. Srb and R.D Owen, *General Genetics* (San Francisco Freeman, 1952)

is still unknown. Such a statement implies that gene action ultimately must be expressible in terms of cell chemistry To get at this problem, we obviously need traits that can be studied biochemically So-called nutritional traits satisfy this requirement. These were first intensively studied in microorganisms, and here we got the first clear answers. To grasp how these experiments were used for the analysis of gene action, however requires some understanding of cellular biochemistry

The basic characteristics of cellular biochemistry pertinent to a discussion of genetics will be briefly reviewed here For a more detailed description, the reader is referred to the book by W D McElroy in this same series.

Comparative biochemistry has taught us that there are great similarities between cells of phylogenetically different organisms. A surprising biochemical unity is, in fact, found throughout the living world. All living cells are similar in composition and contain the same classes of chemical compounds. Despite variation, all cells are built from the same building blocks, and all living things need essentially the same basic nutrients for growth. They require a source of carbon, a source of nitrogen, an energy source, and a source of minerals. From these nutrients, cells synthesize their major chemical components—such as proteins, carbohydrates DNA, RNA, and lipids—as well as the chemical compounds—such as amino acids and purine and pyrimidine bases—which compose these macromolecules.

A cell synthesizes its vital components by a series of well-defined chemical reactions. Most of these reactions require energy A cell, therefore, carries out another series of reactions in which energy can be trapped and forced into synthetic reactions. A constant interplay thus exists between energy releasing and energy requiring reactions. The astonishing fact that the cell can carry out all its necessary reactions under conditions of constant temperature and pressure is due to the presence of specific catalysts, enzymes. (Remember in the laboratory the chemist can use temperatures and pressures over many thousandfold ranges, while the human cell must do its biochemistry at 37 C and a pressure of about 15 lb/in.) Enzymes, like other catalysts, can markedly increase or decrease the rates of reactions without themselves undergoing change. That is why only very small amounts are needed per cell.

All enzymes are proteins, and many of them require a cofactor (vitamin) usually a small molecule or a metal ion, to carry out their catalytic role. Enzymes are also characterized by specificity i.e., a single enzyme can catalyze only a single reaction or class of reactions. In Fig. 15 a series of biosynthetic reactions illustrates these points. The amino acid, arginine, is synthesized from citrulline, citrulline in turn from ornithine and ornithine from glutamic acid. Glutamic acid can be made from glucose by a long

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W.D McElroy *Cellular Physiology and Biochemistry* (Englewood Cliffs, N J Prentice-Hall, Inc., 1961)

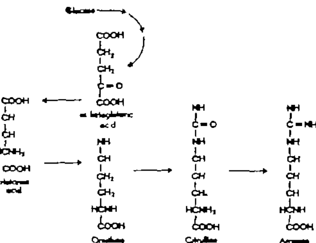


Fig 15. The biochemical reactions required for the formation of arginine from glucose The reactions in the conversion of ornithine to arginine are unique to arginine formation, while the earlier steps are common to the formation of the substances

series of reactions. Thus arginine is formed from glucose by a large number of chemical reactions, and each of these reactions requires the participation of a specific enzyme. A similar series of events is found in the synthesis of the amino acid, tryptophan, although different intermediate compounds are involved.

One last point should be noted. While a majority of the reactions involved in the formation of arginine are common to the formation of many compounds, there are a few terminal steps which are unique. (These will be pointed out shortly) This is generally true of the biosynthetic sequences involved in the formation of most end products

Living cells vary considerably in their synthetic powers depending on which enzymes they possess. Obviously if a cell cannot synthesize a certain essential compound, it requires that compound as a nutrient. Therefore, dietary requirements reflect enzymatic capabilities. Some organisms can synthesize all their amino acids, vitamins, and nucleotides. They get the energy to do this from relatively simple compounds like sugar Many microorganisms are of this type. Plants, however are capable *of synthesizing their amino acids, etc., from even simpler compounds,* but they require sunlight as their energy source. Other organisms lack the necessary enzymes to make many of their essential constituents, and so require them in their diet. Man, for instance, cannot synthesize his own vitamins, and can synthesize only about one-third of his amino acids.

Study of nutritional differences points to an important fact for the study of genetics. Essential compounds, like amino acids and vitamins, are synthesized by a series of well-defined steps. An organism can lose the ability to synthesize such compounds and the loss need not prove fatal, since these substances can be provided in the diet. As a test, then, of whether or not genes control cellular reactions, reactions involving the synthesis of such substances prove to be excellent means of getting at the basis of the genetic control of cellular biochemistry For example, using an organism

which can synthesize all its needed amino acids, we can determine whether a given mutation results in a nutritional requirement for a given amino acid. If it does, does this nutritional requirement induced by mutation result from the loss of ability to carry out a specific biochemical reaction?

Experiments of this sort were initiated by the geneticist, George W Beadle, and the biochemist, Edward L. Tatum, for which they were awarded the Nobel Prize in medicine in 1959 Their experiments with the fungus *Neurospora crassa* gave conclusive proof that mutation can result in nutritional alteration, and that the basis of the nutritional alteration rests on the loss of the organism s ability to carry out specific biochemical reactions.

To see the basis of this conclusion, consider an actual experiment. Neurospora utilizes sucrose as both a carbon and an energy source. It can utilize nitrate as a nitrogen source and requires one vitamin—biotin. Neurospora, therefore, grows on a medium containing sucrose, nitrate, biotin, and minerals (minimal medium) Microconidia (the uninucleate haploid spores of Neurospora) can be treated with a mutagen such as X-rays, and these irradiated spores can then be plated out on an enriched minimal medium, i.e a minimal medium to which the known vitamins and amino acids have been added. This supplementation permits growth and survival of nutritional mutants. Under appropriate conditions, these spores will grow into discrete colonies. As a test of whether or not a mutation which affects a nutritional trait has occurred, an inoculum from each colony can be put into minimal medium. Under these conditions, some of the colonies *are found to* be no longer capable of growing on a *minimal medium,* although they are still capable of growing on a supplemented medium. These strains are found to require one substance in addition to the needed minimal medium. For instance, strains are found which require arginine, others tryptophan, and still others the vitamin, niacin. Treatment with a mutagenic agent can therefore lead to the formation of strains having altered nutritional characteristics.

Are such nutritional differences heritable differences or are they due to a nongenetic or nonchromosomal change? This question can be readily answered by means of the inheritance test. If an arginine-requiring strain ($A-$) is crossed with a parental strain ($A+$) the eight ascospores of a single ascus, representing the four products of meiosis, can be isolated and grown. When these spores are germinated on a medium containing arginine, all eight spores grow If these eight cultures are now transferred to a medium lacking arginine, four of the strains will grow and four will not. Half of the spores are arginine-dependent. This trait, therefore, segregates as predicted for a single-gene difference [refer back to Fig. 11 and in place of (c) substitute ($A-$)] This type of inheritance is characteristic of all nutritional mutants. Alteration of a single gene can result in loss of ability to form a specific essential compound.

Genes and Biochemical Reactions

To pinpoint the biochemical nature of this induced nutritional alter ation, we must look at the cellular reactions involved in the formation of the required end product. Let us continue with arginine The biosynthesis of arginine requires the formation of glutamic acid from glucose. Glutamic acid in turn is converted to arginine through the formation of ornithine and citrulline, reactions unique to the formation of arginine (See Fig. 16.) The synthesis of arginine actually involves the formation of compounds closely related to ornithine and citrulline, but, for the sake of clarity we will discuss its formation as described above. Mutant strains which require arginine for growth can be tested for their ability to grow on these compounds, compounds uniquely required for arginine synthesis. The question, then, is will arginine-requiring mutants grow on ornithine and citrulline as well? (See Fig. 16.) We find that some of the mutants will grow only when supplied with arginine. Others will grow on citrulline, and still others on citrulline or ornithine.

These mutants are not biochemically identical. One class appears to be unable to form arginine because of its inability to carry out the conversion of citrulline to arginine. A second class of mutants appears unable to form arginine due to an inability to carry out the conversion of ornithine to citrulline. They *can*, however form arginine from citrulline, since they can use citrulline for growth. The third class lacks the ability to form arginine, through inability to carry out the conversion of glutamic acid to ornithine Each of these mutants seems unable to carry out one specific biochemical reaction. This suggests that mutation of a *single gene* leads to the loss of the ability to carry out but one biochemical reaction.

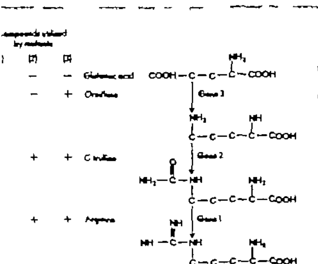


Fig. 16. The geneti con- trol of the biochemical reaction concerned with ar- gi ine formation Com- pounds which ca be used for growth by the various genetic classes re ind cated on the left side.

If these mutants are examined genetically we find that all the strains that cannot convert citrulline to arginine are genetically similar All these strains arise by mutation of the same gene. Similarly all the strains that cannot convert ornithine to citrulline involve mutation of the same gene, but a gene distinct from the one just mentioned. Still a third gene is found in which mutation results in the loss of ability to convert glutamic acid to ornithine. There appears to be a clear association between a specific gene and the ability of the organism to carry out a specific biochemical reaction. This has just been shown for arginine.

Similar results are found for mutations that affect the formation of *tryptophan*, *as well as for mutations that affect the formation of other* vital substances. Not only is there an association between one gene and one biochemical reaction, but there is also specificity a specific gene controls a specific biochemical reaction. Since each of these reactions involves enzyme catalysis, the one–one correlation actually reflects a relationship between genes and enzymes, rather than between genes and biochemical reactions. This correlation established by study of nutritional mutants is known as the one-gene, one-enzyme hypothesis and states that one gene controls the formation of one enzyme. The answer to one question thus shapes another How can a gene control the formation of a specific enzyme? To answer this, we must consider in greater detail the relationship between genes and proteins.

Genes and Enzymes

In the previous chapter it was stated that specific genes control the formation of specific enzymes. This was based largely on *the observation that mutation of a specific* gene may result in the loss of ability to carry out a specific reaction. Additional evidence may be obtained by testing for enzyme activities in the test tube. Cell-free extracts can be prepared both from a mutant strain and the parental strain from which the mutant was derived, and the extracts examined for the presence of the enzyme In general, analysis of the extracts confirms that a mutation which results in the loss of ability to carry out a specific reaction also results in loss of the enzyme activity that is required for the catalysis of the reaction.

This clear relationship between gene and enzyme suggests that an understanding of the nature of this relationship might well go a long way toward explaining how genes act. Let us probe further It would be of interest to know for example, whether the result of mutation is simply a failure to form enzyme or is a consequence of a change in the structure of the formed enzyme. Does a gene exert control by regulating rate and other *quantitative characteristics* of *enzyme* formation, or does it control the architecture of the enzyme, i.e., the sequence of amino acids or their relative proportions? For reasons of experimental expediency these questions have been studied in detail only in microorganisms, and our discussion must deal with these organisms. However it should be borne in mind that

the principles deduced from the study of microorganisms are general and pertain to all living things, including man, as will be documented in a subsequent chapter

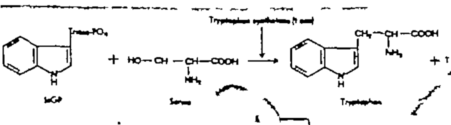
Tryptophan Synthetase

A number of different genes and enzymes have been closely analyzed during the past ten years. Since all the systems suggest a similar relationship of gene to enzyme, we can concentrate on one well-described system. The enzyme, tryptophan synthetase, has been studied in detail in fungi and bacteria. This enzyme is required by these organisms for the synthesis of the amino acid, tryptophan. The formation of tryptophan involves the synthesis of a unique intermediate, indoleglycerol phosphate (InGP in short) The intermediate reacts with serine as shown in Fig 17 A substitution occurs between serine and the triose phosphate portion of InGP to give tryptophan and triose phosphate. This, the terminal step in tryptophan formation, is catalyzed by tryptophan synthetase (t ase)

Mutations affecting the formation of t ase can be recognized by two criteria. (1) they are mutations which result in a nutritional requirement for tryptophan, and (2) for growth, tryptophan cannot be replaced by InGP or its precursor anthranilic acid. With Neurospora, a large number of conidiospores can be exposed to a mutagenic agent, e.g., nitrous acid, and we can select out many independently arising mutant strains of the above type. None of these mutant strains has measurable t ase activity in cell-free extracts. Such an allelic series can be used in a number of ways to examine the nature of the gene-enzyme relationship

One such problem is In how many regions of the entire genetic structure (the genome) of Neurospora can mutation occur and cause loss of t ase activity? Is there a single region—or are there many regions? This problem can be answered by crossing all the mutant strains with one other and determining whether tryptophan-independent progeny arise. If there are two areas on separate chromosomes, or two distinct areas on one chromosome, both of which control t ase formation, frequent tryptophan-independent progeny will be observed as a result of recombination when the strains are crossed. If, on the other hand, the areas are identical or very closely linked, few if any tryptophan-independent

Fig. 17 The reaction catalyzed by tryptophan synthetase.



progeny should be found (see Chapter 3) Crosses between members of the mutant series clearly establish the fact that they are all mutations of the same area of the genome (Fig. 18) In Neurospora, this area is on the second linkage group.

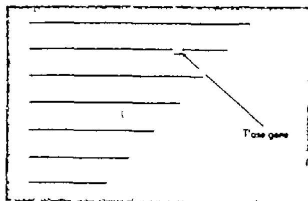


Fig. 18. The chromosomes of Neurospora.

It can be stated categorically then, that there is a single region in the genome which directs T'ase formation, and in which mutation can occur to cause loss of ability to form functional enzyme.

The next problem is Does mutation in this genetic area decrease the rate of formation of the enzyme, or does mutation result in structural changes in the formed protein, or both? This problem can be experimentally examined by determining whether as a consequence of mutation, no enzyme is formed, whether enzyme is slowly formed, or whether the altered gene still directs the formation of a specific protein but one which lacks the catalytic activity of T'ase. To obtain evidence on the last point, we must be able to recognize and pinpoint a given enzyme by properties other than its catalytic activity This can be done by using immunochemical techniques.

Detecting CRM

It has been known for a great many years that animals can form proteins, called antibodies, in response to a challenge by certain foreign materials (antigens) For example, many of you have been immunized against diphtheria and poliomyelitis. You react to the shots by synthesizing antibodies which collect in the serum portion of your blood. If you then come into contact with either diphtheria toxin or the polio virus, an antigen-antibody reaction takes place. The toxin is neutralized and rendered harmless, while the virus is prevented from infecting your nerve cells. Antibody formation can be elicited by many substances, including

proteins. Important to our discussion is the fact that antigen-antibody reactions are highly specific. (Note how specificity is basic to so many biological systems, enzymes, antibodies, viruses) Antibodies formed against one protein will react only with that protein, or proteins which are *structurally related*. It is the specificity of the antigen-antibody response that makes this a remarkably useful tool in studying the consequences of mutation at the enzymatic level.

T'ase is, of course, a protein and it can be purified. If a preparation of purified t ase is injected into rabbits, specific antibodies are formed in response to a challenge by this antigen. These antibodies are present in the rabbit serum and can be recognized by the fact that serum taken from the immunized rabbit completely inhibits catalytic activity when added to the enzyme whereas serum taken from the same rabbit before immunization has no effect on enzyme activity (Fig 19) The antibodies are

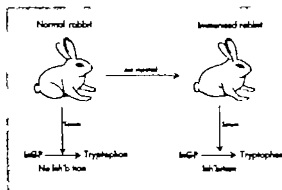


Fig. 19 Enzyme neutralization by antibodies

specific for t ase in that they are without effect on other enzymes. We now have two ways to identify t ase (1) by its ability to catalyze InGP + serine ⟶ tryptophan, and (2) by its ability to react with t ase antibodies. If these two characteristics represent two different properties of the protein, we have a tool that permits the recognition of the t ase protein, even in the absence of catalytic activity

The point of all this is that t ase antibodies can be used to detect the presence of a protein which is structurally similar to t ase, but lacks the catalytic properties of t ase. To repeat, we want to know whether the mutant t ase gene can no longer direct the formation of a protein, or whether it still directs the synthesis of a related but enzymatically inactive protein. This can be examined as follows (see Fig. 20) A cell-free extract of a mutant strain can be prepared. A known amount of t ase antibody is added to the extract. If a protein is present in the extract which can react with these antibodies, it will do so and tie them up. If some t ase

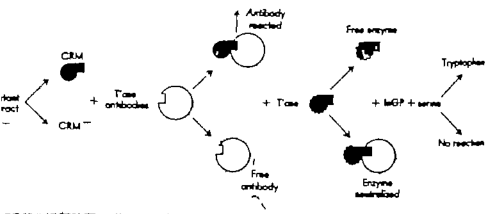


Fig. 20. The test for the presence of protein which is related to the t ase of the parent I strain.

is added, there will be no antibodies to inhibit the enzyme which can then go ahead and make tryptophan from InGP serine, and pyridoxal phosphate. Suppose the cell free extract does not contain a protein which *reacts with the antibodies. The antibodies will,* of course, remain free. They will neutralize added t ase and no tryptophan will be formed from InGP etc. Ultimately therefore, as indicated in Fig. 20, the formation of tryptophan shows the presence of protein in the original cell-free extract which can react with and remove t ase antibodies. No tryptophan formation shows the lack of such a protein in the extract.

T'ase CRM

If t ase-less mutants are examined in this way for the presence of a protein which is serologically related to the parental enzyme, it is found that mutation may give rise to strains unable to form such a protein. Such mutants are called CRM^- (CRM = cross-reacting material) However this is not true of all the mutants. The majority of the mutants do form a protein, and in normal amounts (CRM^+) This protein will react with t ase antibodies but is unable to catalyze the conversion of InGP to tryptophan. Thus mutation may result in loss of catalytic activity without *resulting in loss of ability to form a structurally similar protein. This sug*gests that mutation, in this instance, is not simply controlling the rate of enzyme formation. Rather it is affecting enzyme structure.

This fact can be seen even more convincingly in the following way T'ase catalyzes the reaction InGP and serine ⟶ tryptophan (1) However this same enzyme can catalyze two related reactions, indole and serine ⟶ tryptophan (2) and InGP ⟶ indole (3) The three reactions catalyzed by t ase are shown in Fig. 21 Reactions 1 and 2 both require serine and pyridoxal phosphate, while reaction 3 has no cofactor requirements. If mutation alters enzyme structure, we might predict that

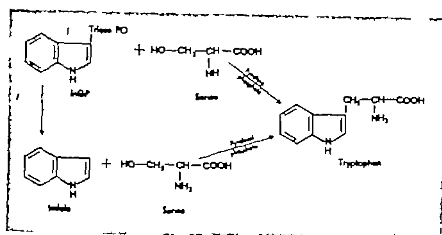


Fig. 21 Th reactions catalyzed by t ase.

mutations of this gene should be found which will result in loss of catalytic activity in one or some combination of these reactions, but not in others, e.g., loss of reactions 1 and 2 but not of 3, or loss of reactions 1 and 3 but not of 2. If the gene, however is controlling enzyme formation by controlling the rate of formation, we would predict that mutation in every case should decrease all three reactions equally

Mutations which result in loss of reactions 1 and 2, but not of 3, are found and easily detected, since such strains characteristically require tryptophan for growth and accumulate indole in their culture medium. All such mutants are CRM+ (i.e., react with t ase antibodies) Their CRM on isolation and purification, can catalyze reaction 3 in the test tube.

Similarly mutations which result in loss of reactions 1 and 3, but not of 2, are also found and again are easily detected, since such strains characteristically require tryptophan for growth, but they can grow equally well if given indole instead. Again, all such mutants are CRM and their CRM, on isolation and purification, will catalyze reaction 2 *in vitro* i.e., outside the cell.

Mutations which result in loss of reactions 2 and 3, but not of 1, have not yet been observed, since reaction 1 is the reaction normally involved in tryptophan formation. Mutations of this class would not require tryptophan for growth and would pass undetected. To pick up a mutant of this kind, we are faced with the alarming prospect of making extracts of thousands of normal strains to find one incapable of carrying out 2 and 3. Similarly mutations which result in loss of reaction 1, but not of 2 and 3, are also difficult to detect.

From such experimental observations, we can conclude that mutations in the *genetic region controlling t'ase formation* result in structural alterations of the formed protein, rather than in quantitative alterations such as rate of enzyme formation. This being true we must conclude that the genetic area in some way controls the structure of the formed enzyme.

Proteins

Tryptophan synthetase, like all proteins, is basically a chain of amino acids. As previously stated, proteins are composed of 20 amino acids in varying amounts, the amounts and their distribution in the chain being

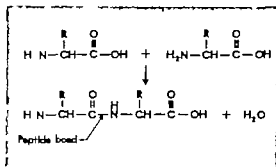


Fig. 22. (Right) The typical bond rmg th mino cid (Bottom) The mino cid sequence f bovine pancreatic bonuclease

characteristic for a given protein. The amino acids in a protein are linked by covalent peptide bonds, and the amino acid sequence is called the primary structure. The amino acid sequence of the enzyme, ribonuclease, which catalyzes the hydrolysis of RNA, is shown in Fig. 22.

The basic or primary structure of all enzymes is made up in a fashion similar to that of RNase. RNase is a relatively small molecule with a molecular weight = 13,683, while the molecular weight of tase is about 150,000. This difference, however simply means that while RNase is composed of 124 amino acid residues, tase is composed of about 1,300. Detailed investigation of proteins suggests that the active catalytic sites of an enzyme consist of only a few amino acids that is, the site on the enzyme surface where InGP combines consists of but a few of the 1,300 amino acid residues. The same is true for the serine site. For catalysis to occur a very specific orientation of these two sites with respect to each other is required. To provide for this an enzyme molecule does not exist as a linear extended structure. Rather each enzyme has a precise folded structure (Fig. 23) Experimental evidence at the present time suggests that the folding characteristics of a protein may be determined by the amino acid sequence. This conclusion may not withstand the test of time but, for the sake of simplicity we will assume that the amino acid sequence does determine the entire three-dimensional structure of an enzyme.



Fig. 23. Model of mole-cule of the protein, myo-globin. This model is based on X-ray crystallographic data. (Courtesy Dr J. C. Kendrew.)

Enzyme Alteration

Since mutations appear to result in alteration of enzyme structure and since enzyme structure in turn is determined by its amino acid sequence, it follows that mutation must alter the amino acid sequence of the product enzyme. Can this be shown? The following discussion will enable us to answer this question.

As mentioned earlier a number of differences can be readily detected among mutants which are all alike in requiring tryptophan for growth and in lacking an enzyme catalytically active in converting InGP to tryptophan. Some of the mutants are CRM− others CRM+ Some of the CRM's catalyze reaction 3 others reaction 2, still others neither Of the CRM's which catalyze reaction 3, some require one cofactor others two, still others none. If we examine the CRM's carefully using quantitative immunochemical techniques, we find differences between them. If we compare them in terms of the temperature that is optimal for their activity we find differences. In fact, after careful study using many diverse criteria of comparison, we are left with the impression that few truly identical alleles are found. Each allele is functionally unique. This means that the genetic area controlling the formation of this enzyme is mutationally complex. It does not consist of a single site, alteration of which produces an identical set of mutants rather the region must consist of a large number of mutational sites alteration of each one of which can produce a slightly different effect.

As we see in Fig 24, we can mentally expand the genetic region and subdivide it into many mutational sites This is what one would predict, if the gene controls the amino acid sequence *and if each mutational site* were to determine one amino acid of the sequence. Mutation could result in alteration of the site in such a way that were the site originally to direct the insertion of valine into the sequence, it might now replace it with lysine. This change in sequence would be expected to have an effect on the folding characteristics of the molecule There would be, as a consequence of this one change, a profound effect on the activity of the product formed. And we would expect a large number of such mutational *sites, and almost endless variation in structure.* This is what is found. To sum up the genetic evidence suggests that the gene controls the amino acid sequence and that mutation results in alteration of sequence.

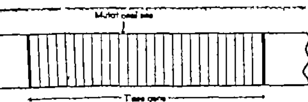
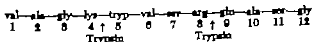


Fig. 24. Mutational sites within the T'ase gene

What do we find if we tackle the same problem by protein chemistry? Can we, by examining mutant protein, actually find amino acid substitution? This is not an easy task. The fact that an enzyme contains a total of several hundred amino acids of twenty different kinds makes the determination of amino acid sequence a particularly thorny problem. The first protein whose entire sequence was determined was insulin. Insulin contains fifty-one amino acids, the final resolution of its sequence took more than ten years, and the work led to a Nobel Prize for the English biochemist, F Sanger Fortunately certain short cuts now permit "fingerprinting" of a protein. These methods enabled Vernon Ingram to demonstrate that there is a single amino acid difference between two, genetically distinct, naturally occurring hemoglobins in man. The finger printing method can be readily shown in the case of RNase.

Fingerprinting

The enzyme trypsin catalyzes the hydrolysis of certain peptide bonds in proteins, namely those in which one of the amino acids in the bond is either lysine or arginine. This action yields protein fragments called polypeptides. Treatment of RNase with trypsin will break the molecule into a number of polypeptides. These can then be separated from each other by a method called paper chromatography The rate of migration on paper is characteristic of each fragment. If, for instance, two different trypsin digests of RNase of bovine origin are chromatographed and the two papers compared, we find similar patterns of superimposable poly peptide spots. If however a digest of bovine RNase is compared with that of horse RNase, the polypeptide spots are not superimposable, indicating that there are differences in the amino acid composition of the fragments and thus differences in the amino acid sequence of cow and horse RNase. Consider the sequence



Treatment of this peptide with trypsin will break it into three frag ments, and, on chromatography we will find three discrete peptides. However if the No. 9 glutamic acid is substituted with glycine, the No. 3 fragment will now occupy a different position on the paper as a consequence of its change in composition. If we compare the chromatographic profiles of these two, we find that the No. 3 peptide of the first prepara tion has disappeared, and we have in place a new fragment at a different spot—an elegant, yet simple method of showing amino acid differences.

A fingerprint comparison of mutant and normal tase has been car ried out by Charles Yanofsky Mutant CRM's from *E. coli* have been purified and subjected to fragmentation by trypsin. The peptide fragments have then been separated from each other by appropriate chromatog

raphy Chromatograms of the parental tase are prepared in exactly the same way and the two fingerprints compared. Some mutant CRM's are found to have fingerprints identical with that of the parental enzyme. More important, however is the fact that several mutants have been found whose fingerprints differ In each instance, the difference is found to involve only one fragment, i.e., the absence of one parental fragment, with the appearance of a new nonparental fragment. The fragment involved is characteristic of the mutant, showing that the change in the amino acid sequence differs in mutants of independent origin.

The fact that a peptide difference is found means that the mutant CRM differs from the parental enzyme in amino acid composition, although the exact difference has still to be determined. A word of caution—the fact that CRM's are found which show no fingerprint difference is not necessarily proof that no chemical difference exists. It simply points out that the difference in these cases may not be detectable by present methods, for the present methods will only detect alterations which result *in a difference in electric charge* or in chromatographic properties. Thus substitution of glutamic acid (a dicarboxylic acid with a negative charge) by lysine (a diamino acid with a positive charge) can be detected, while a substitution of leucine by valine (both uncharged) could not be detected. At present, therefore, cases in which differences can be detected are perhaps more meaningful than cases showing no difference. However we do know that mutation at the tryptophan synthetase locus results in cases of demonstrable fingerprint differences, and we can state with certainty that mutation of this gene results in structural alteration of the formed enzyme because of differences in amino acid sequence. In addition, we know from work with other proteins that mutation at a single mutational site appears to result in the alteration of but one amino acid.

In summary all the present experimental evidence clearly indicates that the genetic region controlling the formation of tryptophan synthetase controls the structural characteristics of this enzyme, i.e., the primary amino acid sequence. It is true for this enzyme, and study of other genes and other enzymes leads to a similar conclusion. One function of genetic material, therefore, involves the determination of enzyme structure. As will be discussed later this conclusion does not force the further conclusion that all genetic material must act in the same way Other genetic areas do play a role in the quantitative regulation of enzyme formation, but most enzymes probably have a gene that acts by controlling structure, and this action of genetic material should thus be explored further

It is now apparent that a gene which controls the structure of an enzyme must be able in some way to serve as an amino acid code. In addition, it must be able to transmit this code to enzyme-forming centers. Since the chemical basis of heredity appears to reside in the compound DNA, we look to the properties and characteristics of DNA for hints to the solution of these new problems.

5

Genes In Action

Researchers have found that DNA can be isolated from cells of nearly all living organisms, and, independent of the source, all DNA is found to have many of the same chemical and physical properties. We no longer doubt that DNA is a vital component of the chemical basis of heredity. However, whether DNA *alone* contains all the genetic information, or whether DNA must act in concert with other material, protein for example, is not as yet known. Final resolution of this problem must come from experiment. To date, no experimental techniques have been devised that permit the complete purification and biological testing of DNA. Moreover, no DNA synthesized in the laboratory has shown any measurable biological activity. More about this later.

The reservation to bear in mind, then, is that DNA, while the major component of genetic material, may not be the only component.

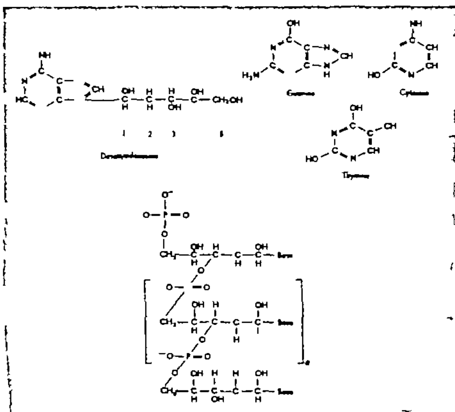
The Structure of DNA

DNA is a macromolecule of variable molecular weight. In general, it is composed of four repeating units called nucleotides. The nucleotides are fairly complicated *molecules* themselves. They all contain phosphoric acid and the 5-carbon sugar deoxyribose. Where they differ is in their third component, the base. There are four bases most frequently found in DNA, two purines (adenine, guanine) and two pyrimidines (cytosine, thymine). T[illegible] individual bases in conjunc-

tion with deoxyribose and phosphoric acid give four nucleotides the purine nucleotides, deoxyadenylic and deoxyguanilic acids, and the pyrimidine nucleotides, deoxythymidilic and deoxycytidylic acids The chemical structures of phosphoric acid, deoxyribose the bases, and one purine nucleotide are given in Fig. 25. Included in the figure is the bond that links one unit in the chain with another This is a phosphoester bond which links the deoxyribose (carbon 3) of one nucleotide to the deoxyribose (carbon 5) of its neighbor The DNA chain is made up of a deoxyribose-phosphate backbone with the bases pointing inward and perpendicular to the fiber axis.

A number of years ago, Irwin Chargaff and his collaborators extracted DNA from a variety of sources and measured the relative proportions of the four nucleotides in the polymer i.e., the base composition. Some interesting results turned up. With any *given nucleotide* (take adenine) the relative amount of this unit in the DNA from, say animal, insect, plant, fungal, and bacterial cells, varied tremendously However the astonishing thing was that with any *given DNA*, if all the purine nucleotides were added up and all the pyrimidine nucleotides added up, the two sums were equal. In addition, it was noted that the number of

Fig. 25. The repeating units of DNA.



deoxyadenylic acid molecules was always equal to the number of deoxythymidylic acid molecules. Similarly deoxyguanylic acid was equal to deoxycytidylic acid. The following is an artificial but illustrative example

	Deoxythymidylic acid	*Deoxycytidylic acid*	*Deoxyadenylic acid*	*Deoxyguanylic acid*
		(Amount of each unit in per cent)		
DNA_1	10	40	10	40
DNA_2	30	20	30	20

The Watson-Crick Model

These observations, together with studies of X-ray diffraction patterns by M.H.F Wilkins, led James Watson and Francis Crick to propose a structure for DNA which is shown in Fig. 26. Watson and Crick suggested that each molecule of *DNA is made up of two intertwined strands, and that* the sequence of bases in one strand determines the sequence of the other the complementary strand, as a consequence of pairing through hydrogen bonding between the purine and pyrimidine bases. Wherever *adenine occurs in one strand, pairing in the complementary strand* is with thymine guanine is paired with cytosine.

The outstanding characteristic of the proposed structure of DNA is that it is double-stranded and that *the two strands are complementary* Extensive physical and chemical investigations support the double-stranded picture of DNA, and our theories on the mode

Fig. 26. Schematic diagram of the W tson-Crick model of DNA. The pairing of the bases by hydrogen bonding indicated.

of replication and mechanism of gene action must now include this fact.

Replication

We know that the genetic material of a given cell must be able to replicate itself exactly The double-stranded nature of DNA provides an attractive hypothesis for the mechanism by which genetic material doubles. Assume that, at replication, the two strands of a molecule of DNA separate, and each single strand maintains its structural integrity Base pairing between adenine and thymine and between guanine and cytosine then implies that each single strand can force the synthesis of an image of itself in the complementary strand. Take a molecule of DNA and label the strands I and II. Normally I and II form a tight couple At replication, I and II separate. Strand I directs the synthesis of a new II similarly II directs the formation of another I The result is two molecules of DNA that are identical with the first. Such a method of replication would permit preservation of identity from generation to generation, for a strand once formed would remain intact and would dictate the structure of its newly formed complementary strand. In addition, if the base sequence were to serve as the hereditary code, we have a way to preserve and transmit the code.

The question now is, what evidence is there that replication in the cell involves a process of strand separation and directed synthesis of complementary strands, with each original strand remaining intact? A number of exciting experiments have recently been carried out to examine these points. A series of studies by Matthew Meselson and Frank Stahl will be described at length to illustrate one kind of experimental attack now being made.

If you look at a table of atomic weights, you will see that nitrogen has an atomic weight close to 14. Most nitrogen atoms do have this weight, but there is a stable (nonradioactive) isotope of nitrogen with an additional neutron in its nucleus and an atomic weight of 15. This is heavy nitrogen, N^{1} Bacterial cells will grow in a medium containing either normal nitrate, $N^{14}O_3^-$ or heavy nitrate, $N^{15}O_3^-$ If the nitrate ion is the only source of nitrogen for the bacterium, the nitrogen atom from NO_3^- *will go into all the molecules that have nitrogen as part of their structure*—in particular the bases of DNA. DNA, extracted from bacteria grown in $N^{15}O_3^-$ will have a higher density weight per unit volume than DNA from bacteria grown in N O $^-$ This difference in density makes it possible to separate "heavy DNA from ordinary DNA in the centrifuge. Under standard conditions, movement of a molecule in a centrifugal field (sedimentation) depends on its density Heavy DNA, with nearly all of its nitrogen as N^{1} will have a higher sedimentation rate and move a greater distance before coming to rest (equilibrium sedimentation = S_e)

than will ordinary DNA. A DNA molecule with half N^{15} and half N^{14} will have an S_e just in between the others.

Meselson and his associates grew cells of the bacterium, *Escherichia coli*, in $N^{15}O_3$ for many generations until virtually all of the DNA contained N^{15} (DNA^{15}) The cells were harvested, put into a medium with *only* $N^{14}O_3^-$ and grown for just that length of time required for each cell to *divide* once. The total number of cells had now doubled, the total amount of DNA had doubled, and each molecule of DNA had doubled. The total amount of DNA^{15} must equal the total amount of normal DNA. A sample of the cells was then taken, the DNA extracted, and S_e measured. It fell just between that of DNA^{15} and normal DNA.

Let us consider the predictions of our original hypothesis on the mechanisms of DNA replication, and the predications derived from a contradictory hypothesis. The original hypothesis was if we start with a cell having DNA both strands are heavy and we have strands I^{15} and II^{15} Let this DNA^{15} replicate in $N^{14}O_3^-$ medium. Each strand remains intact. The complementary strand synthesized by I^{15} is then II^{1} and that of II^{15} is I^{1} The cell divides. One cell gets $I^{15}II$ DNA, the other I^{1} II^{15} DNA. The DNA molecules are identical, each has half of its nitrogen as N^{14} the other half as N^{15} and the S_e of this "hybrid" DNA should be between DNA^{15} and normal DNA.

A contradictory hypothesis is DNA strands do not remain intact. They separate, break down, and the new strands are formed at random from the N^{15} bases contributed by the original DNA and the new N^{1} bases made by the cells from the $N^{14}O_3$ in the medium. True strand I makes a new II and strand II a new I, but now the N^{15} in each DNA molecule is distributed equally between the strands, and we wind up with two DNA molecules, $I^{\frac{1}{2}N15,\frac{1}{2}N1}$ $II^{\frac{1}{2}N15\,\frac{1}{2}N1}$ Remember that before, *one* strand in the DNA molecule had all the N^{15} as in I^{1} II The two types of DNA predicted by the two hypotheses cannot be distinguished by S_e measurements.

Note carefully that while the predicted *distribution* of N^{15} is sharply different, both types of molecules have the same number of N^{15} atoms, and it is the *number* not the distribution, that determines S_e. However *let the bacterial cells go through just one more division in* $N^{14}O$ − *Now* the predictions lead to divergent consequences that are experimentally measurable. According to the second hypothesis, again the DNA breaks down. Since each strand has but one-half of its original N^{15} when it forms *a new strand*, each *new strand* will have one-quarter of the original N^{1} More important, *every* strand will have about the same amount of N^{1}

Consider the consequences of the original hypothesis. After one division, $I^{15}II^{1}$ and $II^{15}I^{1}$ were formed. I^{1} II^{14} in $N^{14}O_3^-$ will now separate and form $I^{15}II^{1}$ and II^{1} I^{1} ! Instead of all the strands being alike, and having one-quarter of the original N^{15} we have two *different* species of

DNA, one with all N^{14} normal DNA, the other species still with one-half its original N^{15} If they exist, they can be separated. The experimental result was that two species of DNA did exist, not one. Moreover the "heavy species contained one-half the original N^{15} content, not one-quarter

This is a beautiful experiment, brilliantly conceived and executed. If you have mastered the concepts, you have a taste of the kind of work going on today in molecular biology Of course the experiment does not provide a final answer It is consistent with, and offers support for our original hypothesis. It disproves the second hypothesis, but there are other possibilities. Why not think one up, and see if you can plan an experiment to test it? (Suppose DNA is not two-stranded, suppose it is four-stranded?)

There is evidence, then, which points to a semiconservative method of DNA replication *in vivo* in the living cell, that is, the physical integrity of a strand once formed is preserved.

Experiments in higher plants also lead to much the same conclusion. One such clever experiment by J Herbert Taylor makes use of thymidine (thymidylic acid without the phosphate acid group) Some of the hydrogen atoms of thymidine are replaced by tritium, which is a hydrogen atom with two additional neutrons in the nucleus. Tritium is radioactive and can take its own photograph when exposed to certain types of film. If root tips of the bean *Vicia faba* are grown in the presence of radioactive thymidine the thymidine is incorporated into DNA as deoxythymidylic acid and is seen to be distributed throughout all the chromosomes If the chromosomes are now permitted to divide once in the *absence* of a radioactive label, we now see one strand which is labeled with radioactivity and one unlabeled strand. The level of organization at which these observations are made is at the chromosomal, not the molecular level. But the observations again point to the conclusion that there is a basic doubleness to the replicating genetic structure, be it a visible chromosome as we find in *Vicia faba* or the DNA molecules we find in bacteria.

Additional supporting evidence for the proposed method of DNA replication comes from enzymatic studies carried out by Arthur Kornberg and his collaborators These workers have shown that DNA, as characterized by its physical and chemical properties, can be synthesized *in vitro* The conditions of synthesis require not only the presence of the nucleotides, an energy source, and a specific enzyme to catalyze the polymerization, but also a small amount of specific single-stranded DNA (double-stranded can also be used but is not as effective) to serve as a primer or initiator (perhaps model" would be appropriate) for the reaction. The DNA formed in the reaction has the physico-chemical properties including base composition, of the primer However biological activity of the source of the primer DNA has not been duplicated. It would be very nice to take transforming DNA from the pneumococcus, make it single-

stranded (you can do this by heating the purified DNA, which serves to separate the strands, and then quickly cooling it to prevent the strands from re-associating) and make more transforming DNA in the test tube. These experiments have not yet succeeded, but they are going on and there are great expectations.

All the contemporary experimental evidence points to the conclusion, therefore, that genetic material is a double-stranded structure and that this structure replicates in a semiconservative way i.e., by separation of the two strands, with each single strand maintaining physical integrity during replication.

While studies on the chemical structure, synthesis and mechanism of replication of DNA are going on, other people are worrying about another problem, the problem of the structure of gene information.

Genes control the sequence of amino acids in enzymes (and probably in all proteins) A part, perhaps all, of the gene is DNA. Therefore, DNA controls the sequence of amino acids in enzymes. DNA consists of four bases. Proteins consist of twenty amino acids How can DNA be coded so that its genetic information can be read off as an amino acid sequence? This is a field in which there are so few facts to work with that speculation is sheer delight.

Cryptography

Again, the cryptographic problem is to work out a four-letter code (the nucleotides) to give a dictionary containing 20 words (the amino acids) Many solutions are possible, and we will describe one resulting primarily from the analyses of Sidney Brenner and Francis Crick.

For ease of discussion, let the nucleotides be the letters *A, B C D* As far as is known, there are no restrictions on base sequence in DNA. We have, therefore, as many repetitions of each letter as we please, and they can be arranged in any order for example, ADCCCCBAAADB One letter is obviously not enough to specify one amino acid, for that would take care of only 4 of the 20 amino acids. A two-letter word is also insufficient. The first letter can be either *A, B C* or *D* That gives four possibilities. The second letter of the word can also be either *A, B C* or *D* This gives $4 \times 4 = 16$ different words (*AA, BC BD DB*) and again this falls short. We try three-letter words. The first letter can be either *A, B C* or *D* as can the second and the third (e.g., *ABC BCA, BDC CDB AAA*) This gives $4 \times 4 \times 4 = 64$ possible words to specify 20 amino acids, an embarrassment of riches. Some restrictions must be placed on which of the words make sense and which are nonsense, to prevent the amino acids from becoming confused.

Another possible source of confusion must be considered with reference to the form of the code. That is, do we want an overlapping code or

DNA, one with all N^{14} normal DNA, the other species still with one-half its original N^{15} If they exist, they can be separated. The experimental result was that two species of DNA did exist, not one. Moreover the "heavy" species contained one-half the original N^{15} content, not one-quarter

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it is clear that we would like to have as mutagens reagents that will cause base substitution.

Ionizing radiations such as X-rays, are highly mutagenic. The effects, in part, are chromosomal and are due to deletions, inversions, and translocations, the latter two being rearrangements of chromosomal material. These occur because X-rays can break chromosomes, some pieces can be lost, while others can recombine but in different order X rays also probably cause base substitution, but this a point that is difficult to prove Ultraviolet light is also highly mutagenic. Like X-rays, ultraviolet radiation can give rise to both chromosomal alterations and point mutations. Again, the molecular basis of the *action of ultraviolet light is not clearly* known.

In recent years, another class of mutagens has appeared and has been found to be of great interest in connection with base substitution and mutation. This is the class of chemical mutagens, one of which is the common inorganic acid, nitrous acid (HNO_2) HNO_2 is a potent oxidizing agent. It can react with primary amino groups, such as are present in the bases, and replace the amino group with an oxygen atom (Fig. 27) As shown in the figure, the reaction of HNO_2 with cytosine yields uracil. HNO_2 can also react with adenine and convert it to a different base, hypoxanthine. Suppose HNO_2 does react with cytosine in a DNA molecule and converts it to uracil. The strand carrying uracil, upon replication, might now make a mistake in pairing, and, rather than pair with guanine, pair with adenine. In a subsequent replication, adenine, in turn, will pair with thymine. HNO_2 will thereby have effected the transition of a guanine-cytosine pair to an adenine-thymine pair thus altering the base sequence of the DNA.

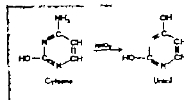


Fig. 27 Oxidative deamination of cytosine by nitrous acid

Nitrous acid was first shown to be mutagenic in tobacco mosaic virus. It is now known to be mutagenic for bacteria, bacterial viruses, and fungi, and it appears likely that it is mutagenic in higher plants as well. Nitrous acid, however does not offer the specificity that one might like, *for it will react with the amino group in the three bases, adenine, guanine,* and cytosine. A technique which, in the long run, may prove of greater value is one involving some trickery Make a compound that looks like one of the bases, but whose behavior is a bit different. Such a compound might hoodwink the cell into incorporating it into DNA, and, once in, it might induce pairing mistakes. These compounds are called base analogues. One compound of this class is 5-bromouracil. It is incorporated into the DNA of bacterial viruses. Normally this base will pair with

adenine, as does thymine. From structural considerations, it is conjectured that 5-bromouracil should also pair with guanine The presence of 5-bromouracil in DNA thereby might enhance the likelihood of transitions of the type guanine/cytosine to adenine/thymine. It is pleasant to report that 5-bromouracil is found to be highly mutagenic. A second base analogue, 2-aminopurine, is also mutagenic. Like 5-bromouracil, it is incorporated into DNA and in this case appears to create transitions of the type adenine-thymine to guanine-cytosine. It is perhaps interesting to note that amino acid analogues are not mutagenic—a fact which permits confidence in the base analogue results.

A curious aside in connection with 2-aminopurine has to do with the phenomenon of mutator genes. Genes have long been known which, when present in the genome, enhance mutation rates of other genes in the organism It has recently been observed that a mutator gene present in a strain of the bacterium *Salmonella typhimurium* has the effect of causing the organism to produce 2-aminopurine, a built-in mutagenic agent.

Extensive investigations by Ernst Freese and Seymour Benzer using base analogues, give strong support to the notion that substitution of one base can cause a mutation In the years ahead, with the acquisition of still better reagents, it may be possible to extend studies of mutation to the molecular level, for it should be possible to obtain reagents which will substitute only for adenine, other reagents specific for thymine, and others for guanine and cytosine Certainly this is one of the promising approaches available.

There is one final question to be considered in this chapter Suppose DNA does determine the amino acid sequence of protein and suppose we do have the correct code How then, is the information transmitted?

Messenger RNA

Here again, the evidence is fragmentary If it were certain that protein synthesis occurs solely in the nucleus, it might be suspected that DNA serves directly as a template (pattern or model) and that the chromosomes were the actual sites of protein formation. Experimental evidence however has revealed that protein formation occurs mainly in the cytoplasm, in specific particulate elements called microsomes. Therefore, we must conclude that an intermediate substance operates between the gene and the protein whose formation the gene controls. The suspect intermediate is RNA.

The second major polynucleotide of living matter RNA, exists in all cells. RNA differs from DNA in its base composition and its pentose, the 5-carbon sugar component. The bases are adenine, guanine, and cytosine as in DNA, but with uracil in place of thymine Its pentose is ribose rather than deoxyribose It has been known for many years that RNA is present

in the cytoplasm as well as in the nucleus and an impressive amount of data has been obtained that implicates RNA in the formation of proteins. Studies on the synthesis of protein *in vitro* further suggest that a number of different RNA s are required as necessary cofactors for protein synthesis. For a detailed discussion of protein synthesis, the reader is referred to the book by W D McElroy in this series.

At this point, we will not concern ourselves with the diversity of RNA s, such as the amino acid-activating RNA, the microsomal RNA, etc., but solely with the relationship of RNA and the nucleus. One solution to the problem at hand would be for the DNA of the nucleus to direct the synthesis of an RNA. This RNA would reflect the specificity of the DNA and would then serve as a messenger in fact, it is referred to as messenger RNA. It would migrate from the nucleus to the cytoplasm and, by interaction with the microsome, serve as a specific enzyme forming site.

The credibility of such a proposal obviously depends on whether RNA synthesis occurs solely in the nucleus. Experiments aimed at answering this question suggest that this may be the case In Neurospora, for instance, in some experiments carried out by Marko Zalokar no cytoplasmic RNA synthesis was observed. RNA was formed in the nucleus, and evidence was obtained that this nuclear RNA migrated out to the microsomes. These observations support the theory that an RNA serves as a messenger between a gene and the site of formation of a specific enzyme. Additional support for this idea comes from current studies of bacteria and bacterial viruses and from the fact that RNA synthesis appears to occur along the chromosome strands. Using an organism that has relatively large chromosomes—cockroaches, for instance—and using tritiated uridine as a visualizing reagent, RNA appears to be formed along the chromosome strands as well as in the nucleolus (a nuclear structure in which it has been thought that RNA is mainly formed) The sum of the evidence favors a messenger RNA, but definite evidence is still awaited.

To sum up we have discussed some aspects of the chemistry of the genetic material, DNA. We have gone over some of the current thoughts on the nature of the code which directs the formation of a specific protein. And we have explored one suggestion on the way genetic information is communicated to the appropriate centers of synthesis From these considerations, we go to the problem of the molecular basis of recombination.

The Molecular Structure of a Gene

In an earlier chapter we introduced and discussed the phenomenon of genetic recombination. Classically recombination refers to the appearance of new combinations of traits in the cell or living organism. The new combinations are formed during meiosis as a consequence either of the random assortment of chromosomes or of genetic exchange produced by crossovers. The following discussion will be concerned only with recombination of linked genetic material.

Breakage Fusion

In meiosis, prior to the first division, intimate pairing of the chromosome strands (chromatids) takes place. Breaks in the strands can then occur and, by reciprocal exchange, chromatid segments can be recombined. This event, of course, permits the recombination of linked genes. For many years, it was thought that such recombination could occur only between genetic regions controlling different traits, but not within a single region itself In large part, this conclusion still holds. It is probable that breakage-fusion recombination does occur primarily if not solely between areas controlling specific traits rather than within a specific region i.e between genes, not within a gene

Although breakage-fusion can be seen to occur the molecular basis of crossing over is unknown. This reflects our ignorance about chromosomal organization in general and, specifically

our ignorance of how the DNA and protein are arranged. However while the problem of chromosomal structure is still largely obscure, a picture is emerging of the structure of the gene. To understand it, we must consider recombination once again.

All the original observations on recombination suggested that no recombination was detectable in matings in which each participant carried an alternative allele of a single gene. With time, exceptions were found. *It is now known that alleles, all concerned with a single function* such as the formation of a particular enzyme, definitely can undergo recombination. Recombination can, therefore, take place within a genetic region performing a single genetic function. The problem is whether this intragenic recombination is a breakage fusion type, similar to that cytologically observed in crossovers between genes, or whether it represents something else. Breakage-fusion would imply that just before reduction division, when the alleles are very closely paired, bonds are broken at identical positions in the DNA molecule of the two alleles and reconnected to the other partners. To maintain the exact nucleotide sequence of both strands, this would have to involve the phosphate ester bond between adjacent nucleotides.

Many difficulties are raised by this suggestion. Do the deoxyribose-phosphate backbones of the DNA chains ever get close enough to enable such bond breakage? Phosphate groups are all negatively charged under the normal physiological conditions of the cell, and if they came close together they would exert strong forces of repulsion. To overcome these forces would require great amounts of energy. One cannot deny that this is possible. The benefits of sex, i.e., recombination, are certainly sufficient for the cell to call on all its resources. Even were this difficulty overcome, a second barrier is inherent in the phosphate ester bond. This is a relatively stable bond and also would require considerable energy to break and reform.

Because of these and other difficulties (for example, consider this problem in relation to coding, use the dictionary to set up some sense alleles and examine the consequences of breakage-fusion) efforts have been made to formulate alternative proposals to account for intragenic recombination. One such proposal is called copy-choice, and the reason for its formulation will become evident when we note some additional curious facts about intragenic recombination.

Consider a cross of two different alleles of gene B: b_1 and b_2. We can represent their position on the chromosomes as

a	$+$	b_1	D
a	$+$	b_1'	D
A	b_2	$+$	d
A	[illegible]	[illegible]	[illegible]

The alleles are symbolized as slightly displaced from one another for the purpose of descriptive clarity Two additional linked genes, *A* and *D* one on either side of *b* are necessary to the analysis. Note carefully that if no crossover occurs between b_1 and b_2, the *b* mutant will be recovered. If a *classical* crossover does occur between b_1 and b_2 normal *B* progeny (*a* + + *d*) will be formed. However crossing over between b_1 and b_2 must also yield recombination of the outside markers, that is, *B* progeny should also be mutant for *a* and *d* The expected reciprocal recombinant class is *AbD* mutant *b* and wild type *A* and *D*

Actually in many crosses of this type, one finds normal *B* progeny which are unexpectedly normal for *A* and *D* as well (genotypically *ABD*) In addition, in intragenic crosses there are cases in which it has been possible to prove that one of the two recombinant classes never was formed, that is, either *aBd*, or *Ab b D* was formed, but not both. This is referred to as nonreciprocal recombination.

The fact is then, that nonreciprocal recombination does take place within the gene. This means that nonreciprocal recombination presumably takes place between DNA molecules To account for this, breakage-fusion in DNA, although certainly possible is unlikely ergo, copy-choice.

Copy-choice

Copy-choice is assumed to work in the following manner Prior to replication, the two strands of DNA uncoil to form single strands. (This occurs through as yet unidentified physical forces or is catalyzed by the enzyme ravelase) Single-stranded DNA then replicates its complementary strand. An error in the replication could occur as is shown in Fig. 28. Suppose a strand, complementary to strand A, were in the process of replication. Assume that another strand, identical to A except for an alternative allele (strand *a*) were also in the cell and very close to the newly forming complementary strand. The replicating strand, on rare occasions, might switch and use strand *a* as its model for a small region. The replicate, then, would not mirror the parental strand, but would effectively recombine the genetic codes of the two strands. With this model, there is no need for breakage-fusion or the formation of reciprocal classes. This type of copy-choice replication also would explain the recombinational events observed in bacterial viruses or in bacterial transformation.

In bacterial transformation for example we know that a small amount of DNA is taken into a cell, and that a portion of the code it carries, in some way can become incorporated into the replicating code of the cell. In this case, the copy-choice mechanism suggests that the incorporated DNA pairs with a homologous region of the parental strand and, as a consequence of a copy-choice error in replication, recombination of the traits of the donor DNA and the parental DNA occurs

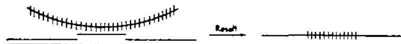


Fig. 28. Copy-choice recombination. The two parent I strands are homologous but not identical.

Whether recombination occurs by copy-choice is unproved. But the facts are clear. Within one chromosome, at least two types of recombination must occur: recombination between genes and recombination within genes. The mechanisms of recombination still await clarification.

Since recombination within a gene does occur, we can use it to map a gene, and to try and see what its fine structure is. Contemporary fine-structure analysis has been provided by Seymour Benzer, who has also enriched our genetic vocabulary with the terms "muton," "recon," and "cistron," the units of mutation, of recombination, and of function, respectively. Benzer's experiments have been carried out with a bacterial virus, and he has gone far toward defining genetic units at a molecular level. Since we have previously and at length described the genetics of tryptophan synthetase formation, we will continue to use this gene as a model in describing genetic fine structure.

Fine Structure

As we discussed in Chapter 4, an allelic series of tryptophan synthetase mutants can readily be isolated. These are mutations of a single gene on chromosome 2 of Neurospora. These allelic strains, if crossed with one another, do yield infrequent tryptophan-independent progeny, and now we can map the genetic region by determining the frequency with which different allelic crosses give rise to tryptophan-independent progeny. The map can be prepared by using this one criterion and without attempting the almost superhuman task of determining whether the reciprocal class is also found, that is, the gene with the double lesion!

As was also discussed in Chapter 4, we know that many functional differences can be observed between the members of this allelic series. We can set up a broad classification of these mutants in the following way (Fig. 29). Mutations may give rise to either a CRM+ or a CRM− strain. The CRM+'s, as indicated in the chart, can be subdivided further into three classes: (I) strains which have lost catalytic activity for all three reactions catalyzed by the parental enzyme; (II) strains without catalytic activity for reactions 1 and 2 but with activity for reaction 3; (III) strains without activity for reactions 1 and 3 but with activity for reaction 2. Class II can be subdivided further in that some of these mutants have no cofactor requirement for reaction 3, others require, in contrast to the parental strain, pyridoxal phosphate, and still others require both B6 and the amino acid serine.

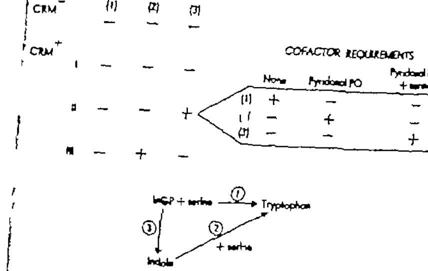


Fig. 29 Classes of t ase mut ts.

From this information, it is apparent that the genetic region co trolling the formation of tryptophan synthetase is highly complex. I analyzing the fine structure of the region, therefore, we can subdivid the region into a large number of individual mutational sites. Such muta tional subdivisions of a functional area might be considered as *mutons* A muton, as defined by Benzer is the smallest element that when altered can give rise to a mutant form. A single functional area, a gene, consists of many mutons. Since the gene is DNA, it is reasonable to inquire what the muton represents in terms of nucleotides. A muton should represent the minimum number of nucleotides that must be altered to permit amino acid alteration. From his analysis of bacterial virus recombination, knowledge of the total amount of DNA per virus, and some ingenious assumptions, Benzer estimates that at maximum a muton consists of a few about three or four nucleotides and hopes it is really one. (This would fit our code.)

What about the structure of a gene in terms of recombination? Benzer suggested the term *recon* to define the smallest unit that is interchangeable by genetic recombination. The analysis suggests that the recon may have the molecular dimensions of one nucleotide. As mentioned, if various tryptophan-requiring mutant strains are crossed with one another tryptophan-independent progeny occasionally arise by intra genic recombination. Using orthodox mapping procedures, we can now map the mutons. It turns out that mutons appear to be arranged in a linear array within the functional gene area (*Fig. 30*) Within the area, certain regions are easily susceptible to mutation (noisy regions) other regions are impervious to mutation (silent regions) An additional observation of interest is that alleles that are functionally similar are not scattered hroughout the genet, but are clustered. In the genetic

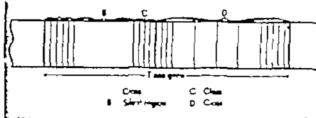


Fig. 20. Schematic representation of the genetic fine structure of the t ase gene. For class type, see Fig. 2?

area of tryptophan synthetase, mutations of class I appear to be clustered in the middle of the genetic area. Mutations of class II are found huddled together on the right side and mutations of class III on the left side. These clusters of functionally related, highly strung and highly sensitive areas respond sufficiently to mutation to be picked up as functional variants.

What about the intervening regions of silence? Is it more difficult to obtain mutation there? Or are experimental tools still inadequate to resolve the effects of mutation in these regions? This is related to the problem of knowing what to look for and while there are no answers to the questions posed, there is a suggestion. It may be that mutation is just as frequent in the silent regions as in the noisy regions. This would imply that in certain areas of the enzyme, amino acid substitution can alter enzyme activity while in other areas substitution cannot. To actually find a mutation that has no functional effect on the enzyme, we may merely have to grow up a few million normal-appearing strains, purify and fingerprint the enzyme, and see what happens. Either that or have a clever idea. The problem waits.

Some interesting evidence for the above concept, however comes from the superb structural analysis of the protein myoglobin carried out by Dr J. C. Kendrew and his associates. By X-ray crystallographic diffraction and the liveliest imagination, it was possible to determine the entire structure of myoglobin. The structure is given in Fig. 23. In their work, these authors achieved a resolution sufficient to let them look at the folds within the protein chain. One fact emerges clearly—the protein molecule is folded in such a manner as to bring into close spatial relationship amino acid groups relatively distant from one another in the chain. Thus we find areas in which folds occur as well as areas of straight protein chain.

The importance of these structural considerations becomes immediately apparent, since there is a great deal of evidence available that permits the extension of the picture of myoglobin to other proteins, including enzymes. If portions of neighboring chains of amino acids, brought together by folding, constitute sites of enzymatic activity and other proteins of the molecule, the straight sections, represent only regions of structural support, then a reasonable correspondence can be et up on the one hand between the noisy regions of the gene and the sites of enzymatic activity and on the other hand between the quiet regions and the areas of straight-chain structural upport. In the latter case, substi tion of one amino acid for another bro nt by a mutation in

quiet region easily could have little or no measurable effect on enzyme activity. Mutation in the noisy region, with consequent substitution of an amino acid in the site of enzyme activity could have a dramatic, easily measurable effect.

At the present time, therefore, we can state only that there is still much to be learned about the nature of protein folding, what directs it, and whether the gene affects folding apart from sequence determination. It must also be emphasized that while noisy and silent regions have been found in the genetic fine structure of tryptophan synthetase, similar regions have not been observed by Benzer in the genetic fine structure of the trait he studied in a bacterial virus. The reasons for these differences await reconciliation. This is an area in which many experiments are now being carried out, and in which the combined use of biological and sophisticated chemical techniques are being profitably exploited to gain further insights into the molecular basis of heredity.

Complementation

We now come to an additional and intriguing question. Must all the mutons of a single genetic area be present together and in a prescribed order for a specific enzyme to be formed? We can find out by making use of a test called the cis/trans test, a test which gave rise to the unit of function, the *cistron*. The principle of this test is illustrated in Fig. 31.

Basically the problem is whether enzyme formation will occur when all the necessary genetic elements are present, not in a single linkage group (the cis position) but distributed either between two homologous chromosomes located in the same nucleus or in two different nuclei carried in a common cytoplasm (the trans position). Surprisingly it appears that all the genetic elements necessary for enzyme formation need not be bound together on the same chromosome. The term *cistron* will be used to define the smallest subset of mutons that must be present together in the cis position, for enzyme synthesis to occur in the cell. (We are deliberately simplifying the original definition as given by Benzer but at no sacrifice for the exposition of the general concept.) As an illustration, if there are 100 mutons in a gene, assume that the presence of *at least* 20 linked mutons on one of the chromosomes is necessary for enzyme formation. A partition of 19, 81 on one allele and 17, 83 on the other would not permit enzyme formation. A partition of 19, 81 and 21, 79 would permit complementation.

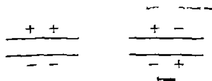


Fig. 31 The cis/trans test. Object: Will the trait be manifested in the trans configuration, or only in the cis configuration? The same genetic elements are present in both cases, but differently distributed.

Let us try the following type of experiment. If two strains of Neurospora of similar mating type are grown together fusion of hyphae between the strains can occur and new hyphae will be formed that carry the two parental nuclei in a common cytoplasm. The nuclei do *not* fuse to form a sexual phase but divide independently of each other This fusion strain is called a heterocaryon and can be seen easily if we grow a strain of Neurospora which requires tryptophan together with a strain requiring arginine. If the growth medium contains neither tryptophan nor arginine, a heterocaryon will be formed. These strains can grow because the nuclei that are unable to form arginine carry a gene which enables them to make tryptophan, and the tryptophan-requiring nuclei are genetically capable of forming arginine. Thus we have a sort of offbeat heterozygote, since the strains have all the necessary elements for tryptophan and arginine formation, but distributed between two different nuclei, rather than present in one nucleus. These two nuclear types together get along as well as the wild type by itself These two nuclear types are said to complement each other

A similar complementation test can be carried out using two allelic tryptophan synthetase less (t ase-less) mutants. As a test of complementation, we can determine whether a heterocaryon formed between two t ase less mutants in our allelic series is capable of growing on a medium lacking tryptophan. If it is, can such a strain form t ase?

Certain combinations of alleles do exactly this. The heterocaryon can grow in the absence of tryptophan and can form t ase. In this case, the heterocaryons grow slowly and the amount of enzyme formed is small, but it is an experimental fact that complementation does occur between certain alleles and does result in enzyme formation. It is even possible to map alleles in terms of complementation by testing all the mutants in our extensive allelic series against one another The experimental result is that the relative position of alleles in a complementation map is similar to their order in a recombination map but the correspondence is not identical. These two maps must then tell us different things—but research on this recent concept has not yet advanced to the point where the difference is understood.

But one thing has been learned. Such complementation maps tell us that alleles of similar function do not complement. That is, alleles all belonging to class II, those without enzymatic activity for reactions 1 and 2 but with activity for reaction 3, will not form tryptophan-independent heterocaryons. However by this test, members of class II will complement members of class III, mutants with no catalytic activity for reactions 1 and 3 but activity for reaction 2. Can we explain the type of interaction involved in complementation? No. But we can speculate

We know that many enzymes consist of at least two independent polypeptide chains linked together by covalent bonds (e g., S-S bridges) and by attraction between electrically charged groups. These molecules

can dissociate. Genetic analysis tells us that a gene consists of relatively few cistrons. At present, we are tempted to predict that a cistron may represent the genetic area that controls the formation of a single polypeptide chain. If an enzyme consists of more than one polypeptide, the functional genetic area defined by the gene will then consist of an equal number of cistrons. But this is just a reasonable guess. The statement probably will not survive long, and may not survive publication.

Summary

We now can arrive at the following conclusions concerning a gene. A gene is the area in the chromosome in which mutation can occur and affect the ability of the organism to form a specific enzyme. This genetic area is a complex region in terms of both mutation and recombination. It can be subdivided into many mutons and recons and into a few cistrons. We might now define, or perhaps we should say redefine, the gene as the entire functional area required for the formation of a specific enzymatic activity. The astute reader will realize that the definition is uncertain. *In the years ahead, it is expected that the definition of a gene will continue to change,* for the gene is essentially an operational unit. In the hands of earlier workers, it was first a unit of segregation and then a unit of classical recombination. At present, it is a unit of function, and in the future, it may well be a unit of DNA. In any event, we are now beginning to talk about the molecular dimensions of units of heredity.

Although DNA appears to be the substance that transmits hereditary information, we have not mentioned its organization in chromosomes. The visible chromosome of higher forms probably contains all the DNA of the cell. The inheritance test indicates that genes are arranged in a linear array in the chromosomes of all organisms from bacteria to man. However, the linear array can obviously take many different forms. We know that the DNA, in some way, must be condensed in chromosomes, since the total length of DNA, calculated from the Watson-Crick model, far exceeds the actual chromosome length. It has been suggested that the DNA is arranged in Christmas-tree style, with the DNA as limbs along a protein trunk. Reciprocal recombination then could occur along the trunk and *nonreciprocal recombination along the limbs, but again this is* only speculation. The hard facts are still to be discovered.

Genetic Mechanisms

In the preceding chapter, our emphasis has been on the gene itself, its structure and its function. We now turn to a consideration of the mechanics of gene transmission and the laws of heredity. We introduce this subject by again invoking the co-operation of our favorite haploid organism, *Neurospora crassa*. In Neurospora, the vegetative transmission of traits takes place exclusively by mitotic nuclear divisions. As the organism grows, nuclei divide, and as the number of nuclei increases, the mycelial mass expands. As we already know in a well-behaved mitotic division, without chromosomal imbalance or gene mutation, two daughter nuclei are formed identical to the parent nucleus.

Since the growth phase of Neurospora is haploid, each nucleus contains only one set of chromosomes. Each chromosome differs from all the others in the genes it carries and, therefore, in the set of traits it determines. When gene mutation occurs in any haploid nucleus, an immediate alteration of gene function in that nucleus must take place.

Mutation and Selection

Whether or not such a nucleus survives depends on many factors. To illustrate, assume that we have two Neurospora conidiospores, each containing one identical nucleus. They differ, however in a single respect. In one of the spores, a mutation of the tryptophan synthetase gene has occurred, inducing a nutritional

requirement for tryptophan. If we now place both spores in an environment that normally permits germination but that contains no available source of tryptophan, what happens is easily predictable. The normal spore will germinate to give rise to a new plant, the mutant spore will not germinate, and its nucleus with its mutant gene, will be lost.

Consider another type of mutational event. Assume that a single mutation occurs in one spore to confer upon it the capacity to germinate and to grow more rapidly in a relatively arid environment. If the two spores are placed in a normal environment but with a climate in which the moisture content is reduced, the mutant spore will germinate and the plant will grow faster than the normal spore The mutant will extract and utilize all the resources of the environment at a more rapid rate than will the normal plant. In sum, the mutant will outgrow the normal plant and displace it from the new environment. In this case, the normal nucleus with the normal gene will be eliminated.

In the above examples, one or the other of the parent and mutant nuclei was eliminated. These examples, although somewhat simplified, illustrate a principle that has been validated by countless observations and experiments. Mutation and competition in the original or in a new environment represent powerful forces in the creation and survival of new fitter genotypes in a haploid organism.

Meiotic genetic recombination also occurs in Neurospora, since there is a sexual phase. (Note that in Neurospora, the diploid stage is transient and gives rise immediately to the haploid plant, in humans, the complete reverse is true.) To form a sexual zygote, two cells from strains of opposite mating fuse and form a diploid cell. Each of the haploid nuclei contributes a set of haploid chromosomes and a cell containing two such sets is formed. Such a cell is diploid. It contains two genes that govern any specific trait. If the two sets of chromosomes are identical in gene content, the diploid cell is called *homozygous* but if the sets differ—that is, if there are alternative alleles of certain genes in the two sets of chromosomes—the diploid cell is termed *heterozygous* The terms homozygous and heterozygous can also refer to a gene A diploid cell with two identical alleles is homozygous for that particular gene if the alleles differ the cell is heterozygous for the gene.

As we mentioned before, meiosis permits reassortment of chromosomes. It also permits crossing over between homologous chromosomes. Meiosis, therefore, is a sexual mechanism for the recombination of genes contributed by the parental strains. It permits the ready formation and transmission of new gene combinations. We might say then, that the life cycle of Neurospora permits the evolution of new types by at least two mechanisms. New genes with new functions can arise in the haploid organism and be immediately expressed in its uninucleate conidiospores the second mechanism permits recombination of these new genes with the genes already present All these can then be subjected to the test of

the environment, which can select out those genotypes best fitted for the environment.

Bacterial Recombination

We have shown how new gene combinations form by genetic recombination in an organism that enjoys meiosis. The inquiring mind, however might wonder about those organisms that spend their entire life as haploids, organisms such as bacteria and bacterial viruses. Can organisms such as these also undergo genetic recombination or must they evolve new genotypes by mutation alone? Experimental work of the past decade has shown that bacterial genes are linked and must therefore be organized in structures that are similar to the structure of a chromosome. This fact has been deduced from genetic experiments, although it has not yet been confirmed by cytological examination.

Escherichia coli and *Salmonella typhimurium* are examples of two bacterial species which characteristically contain one linkage group and are haploid. Vegetative reproduction takes place through duplication of the bacterial chromosome and the transmission of an identical chromosome to each daughter cell. We suspect that this event is similar to mitosis, although this conclusion lacks cytological confirmation.

As we will discuss in a moment, mutation plays a major role in the evolution of new genotypes in these organisms. However bacteria are also capable of recombining the genetic traits of two separate cells. In bacterial transformation (see Chapter 2) certain bacteria can take up purified DNA from a genotypically different cell, and the new information carried by the DNA can be incorporated into the recipient cell's own genetic apparatus and transmitted to its progeny. In transformation, therefore, a cell is formed that can be called, with some charity heterozygous. Note that it is heterozygous for only a small region of the genetic structure, in other words, a partial heterozygote. Unlike the formation of a diploid cell in Neurospora, in transformation the genetic contribution of the two parental cell lines is unequal, and recombination may well result from a copy choice type of replication.

Regardless of the mechanism involved, however recombination such as that described for bacterial transformation clearly does occur and the formation of partial heterozygotes is characteristic of genetic recombination in the bacterial world in general. It is the *manner* in which donor DNA is acquired by a recipient cell that varies. In transformation, the donor cell DNA is picked up from the medium. Another mechanism for recombination, bacterial conjugation, was discovered by Joshua Lederberg in certain strains in *E. coli*. In these strains, two cells can fuse and form a cytoplasmic bridge. The chromosome of the donor cell is then injected into the recipient cell. Bacterial conjugation requires a mating-type system, since cells of only one kind (Hfr) can serve as donors, while

other cells (F^-) can serve as recipients. The difference between (Hfr) and (F^-) cells is still a mystery. Surprisingly enough, even under conditions in which a cytoplasmic bridge is formed between the two cells, the entire genome of the donor cell is seldom injected. Again, the recipient cell is only partially diploid. After conjugation, the cells separate, and recombination takes place between the genes injected by the donor cell and the genes present in the recipient chromosome. Daughter cells bearing new combinations of the two parent cells are formed, and the new combinations are transmitted to their progeny.

Still a third mechanism for recombination, bacterial transduction, exists in bacteria. Bacterial transduction differs from the other mechanisms in that the agent for transmitting a portion of the DNA from one cell to another is a virus. It may be somewhat disquieting to think that a virus, the very model of a cell parasite, can transfer its host's DNA from one cell to another. It does occur, however, even if unexpected. The story is briefly this. While many bacterial viruses appear to infect and invariably destroy the specific bacterium they parasitize, other viruses behave differently. These can and do infect and destroy, but, under some unexplained circumstances, they infect cells and then turn benign. The viral DNA becomes attached to the chromosome of the host cell and multiplies when the host chromosome multiplies. If a virus has this characteristic, it is known as a temperate virus. The condition whereby the viral DNA sits quietly on the host chromosome is called *lysogeny*.

From time to time, the virus spontaneously erupts (by now you realize that "spontaneous" in biology means "we do not know why"), reassumes its malignant form, multiplies, destroys its host cell, and pours out ready to infect another host cell, prepared either to destroy it or to lysogenize. When the DNA of a temperate virus in a lysogenic condition is activated, it forms a complete virus. It can then lysogenize a new host and carry a piece of its old host's chromosome into the new host. Moreover, the genetic information of this chromosomal fragment can become incorporated into the new host.

Bacterial recombination, therefore, characteristically takes place through a number of different mechanisms, but only partial diploid cells are formed, and copy-choice may be the primary mechanism for the incorporation of the new information. The absence of equal genetic contribution and the absence of a true reduction division distinguish bacterial recombination from that found in higher organisms.

One of the many enigmas that surround these various forms of bacterial recombination is the role they play in nature. Do they represent laboratory oddities forced upon nature by the investigator or are they crucial to the survival of cells? And of equal interest, are these exclusively bacterial phenomena? We know that human cells are susceptible to certain types of virus and that they can also harbor viruses for years, a condition analogous to lysogeny. Do human viruses carry DNA from cell to

cell and, if they do, why? These puzzlements are not meant to glorify the status of the question mark, but to give you some idea of the things that intrigue biologists at the moment, and of the things they are trying to learn. Therefore, although we know that bacterial recombination can occur its role in the creation of new bacterial types has still to be assessed.

At the present time, it would appear that mutation and selection are the major forces involved in the evolution of new bacterial cell types. We know that mutation occurs spontaneously and in all genes that have been studied, although the spontaneous rate of mutation among genes appears to vary In general, bacteria are single haploid cells. Mutations are rapidly expressed. Moreover haploid organisms multiply very rapidly as compared with the higher diploid forms. In the laboratory under suitable conditions, the bacterium, *E. coli*, will divide about every half hour At the end of 15 hours, one cell will give rise to 10^9 or about one billion cells. This means that mutant populations can easily be expressed, and selection of new genotypes by the environment can easily occur We have illustrated the principle of mutation and selection earlier in the chapter and will now discuss one additional example which has come to our attention rather forcefully in recent years.

Escherichia coli is ordinarily killed by the antibiotic substance, streptomycin. If streptomycin is added to a culture of sensitive cells, the cells will be killed. However *E. coli* contains a gene which can undergo mutation and confer resistance to streptomycin upon cells that carry it. Mutation to streptomycin resistance occurs spontaneously about once in every 10^8 to 10^9 cell divisions. In the absence of streptomycin in the environment, a *mutation from streptomycin sensitivity to streptomycin resistance* will go unnoticed by us, and apparently also by nature, since the streptomycin-resistant genotype does not accumulate to any noticeable extent in normal populations of *E. coli*. In the presence of streptomycin, however only those cells with the resistant gene can survive and grow and the streptomycin-sensitive population is eradicated. In a world filled with streptomycin, the mutant gene becomes the normal gene.

This is an important and contemporary problem. The microorganism has a remarkable genetic flexibility the potential to mutate and meet the challenge of as yet unknown environments in order to survive. In a similar way as you must know the flies have conquered DDT and now man is considering whether he can conquer *the ionizing radiations* and the weightlessness and the new challenges of space.

Diploidy

The laws of genetics were formulated from the study of higher plants and animals. These organisms differ from fungi and bacteria in that they are *diploid* and *invariably* possess some form of sexual reproduction. Typically a new individual arises by fusion of two nonidentical gametic

cells, the egg and the sperm cell. Fusion results in the formation of a diploid cell, which, by division, growth, and differentiation, gives rise to a new adult. Reproduction, therefore, requires the formation of haploid gametes from the diploid cells of a mature organism. The formation of progeny consists of two meiotic divisions, one in the male and one in the female cell lines The fact that in the diploid type of life cycle a new individual arises by fusion of two nonidentical gametic cells suggests that recombination plays a major role in the formation of new types.

The formal genetics of diploid organisms differs in certain respects from the genetics of haploid organisms. In diploid genetics, the observable *characteristics of the organism (the phenotype)* derive from the existence and expression in each cell of a duplicate set of genes. Further allelism implies that for any given gene a heterozygous condition can exist. This raises the problem of the phenotypic expression in the organism of more than one allele To illustrate this problem, let us choose a microorganism, a yeast, whose vegetative growth phase is diploid.

Dominance

Diploid yeast cells divide by mitosis and can also undergo meiosis to form haploid cells The fusion of two haploid cells results in the re-establishment of the diploid line. Two haploid strains both capable of forming tryptophan synthetase (tase+) upon fusion, form a diploid cell carrying two tase+ genes. Two haploid strains incapable of forming the enzyme (tase−) fuse to give a diploid cell line with a nutritional requirement for tryptophan. In a fusion between a tase− strain and a tase+ strain, the diploid line now carries both alleles, on one chromosome is a gene that can direct the formation of the enzyme, while on the homologous chromosome is a mutant allele that cannot. The phenotype of the diploid cell line, *which is heterozygous for the tryptophan synthetase gene, is* tryptophan independent. The cells synthesize the enzyme and can grow in the absence of added tryptophan The tase+ gene is said to be dominant over the tase− gene

In general, the ability to synthesize an active enzyme qualifies a gene as dominant. The presence of a dominant gene in the diploid cell also usually permits the formation of a specific enzyme at a rate sufficient to allow growth, although not necessarily at the maximum possible rate. This example illustrates the problems, however that can arise as a consequence of heterozygosity For instance, a mutation in the tase+ gene can result in the formation of a mutant enzyme that might in some way inhibit the normal enzyme. This would lead to a requirement for tryptophan in a heterozygote containing a normal tase+ allele In the absence of other knowledge, *we would then conclude that the mutant gene was dominant* to the normal allele.

The relationship of dominance and its converse, recessiveness, is a

consequence of the fact that in diploid cells, each one of the alleles exerts its own action and the phenotype must reflect the result of their interaction. If one of the alleles can function and the second allele cannot, and is neutral, the *former would appear dominant. If the second allele inhibits* the action of the first, the second would appear dominant. If one allele can direct the formation of a product but in insufficient amount, the heterozygote appears intermediate, and dominance is indeterminate.

Many interactions are possible and all are found. On the level of the organism, a homozygotic organism is, by definition, one in which all the gene pairs are identical, while a heterozygous organism is one in which a variable number of gene pairs differ All gradations between complete homozygosity and complete heterozygosity exist; and allelic interaction is a problem basic to all of diploid genetics.

The fact that a vegetative cell carries duplicate genes confers genetic stability on a cell, and thus may be one of the reasons that the majority of higher plants and animals are diploid. In haploid organisms, such as Neurospora and bacteria, mutation can lead to the rapid phenotypic expression of the mutant allele In a diploid organism, however expression of a recessive mutation requires that the mutation occur at least twice, either in both members of a diploid pair or singly in two diploid genomes followed by recombination. If the probability of mutation of one member of a gene pair is 1 in 10^7 cell divisions, the probability that both members of the gene pair will mutate in the same cell at the same time is a product *of this probability or 1 in* 10^{14} *cell divisions. This is a rare event. In* addition, if the mutation occurs separately the mutant alleles must find each other or they may be lost in the meiotic shuffle.

Thus many mutations can occur in a diploid organism and pass undetected during the vegetative propagation of this organism. The strain can simply grow and accumulate recessive mutations. Occasionally a double recessive will be formed, and the phenotypic expression of the gene can then be observed. If it is valuable, we treat the organism and the parent lines with great deference. If it is troublesome, we try to correct or eliminate it. These are common practices in horticulture and animal husbandry

While diploidy confers great stability on vegetative reproduction, sex combined with diploidy provides the opportunity for a species to try out many new gene combinations To see how this works, let us consider *the transmission of traits in a diploid organism in which the two diploid* cell lines both undergo meiosis and form haploid gametes.

Three:One

Assume that two cell lines differ in a particular trait, enzyme formation, and that a dominance relation holds. Let *E* represent the (dominant) gene which directs enzyme formation (enz^+) in the phenotype, and *e*

represent the (recessive) inert allele. Assume that one organism has the genotype *EE* and the other *ee*. A cross between the two will give the results outlined in Figure 32A, where *P* stands for parent, G_m for gamete produced by meiosis, and F_1 and F_2 for the first and second filial generations. In this case each parent produces but one type of gamete, and all the F_1 progeny are alike, *Ee* (enz+).

A cross of two F_1 organisms is diagrammed in Fig. 32B. Here, each F_1 gives rise to two gametes. These are usually produced in equal number. Random fertilization means that fusion of any gamete from one cell line by any gamete of any other cell line is equally probable. Random fertilization of F_1 gametes produces three F_2 genotypes in the ratio shown in the figure. Since both *EE* and *Ee* are (enz+) there are only *two* phenotypes in 3:1 ratio. In the F_2, note the reappearance of the two parent types, *EE* and *ee*, and the persistence of the recombinant type, *Ee*. In diploid genetics, a 3:1 phenotypic ratio in the F_2 is a clear indication of a single-gene difference.

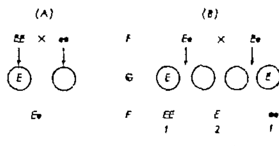


Fig. 32. The inheritance of single gene. (A) Mating of homozygous dominant and homozygous recessive. (B) Mating of F_1 heterozygotes.

Nine:Three:Three:One

Let us now analyze a cross involving two gene differences. Since in this book we love enzymes, let the traits controlled by the genes be $T =$ the presence of tase (tase+), $t =$ no tase (tase−), $H =$ the presence of *histidase* (*hase*+), $h =$ *no hase* (*hase*−). *Assume that the T and H* genes are on separate chromosomes.

The results of a cross between the parental types, *TTHH* × *tthh*, are given in Fig. 33, as is the result of the F_1 cross. By now you have all the information necessary to calculate and confirm the ratios and the genotypes given in Fig. 33. Moreover, you can backcross the F_1 to either parent and predict the results. You can check your answers by referring to any of the standard textbooks given in the bibliography. The appearance of a 9:3:3:1 ratio (with dominance in each gene) clearly indicates the segregation of two unlinked genes.

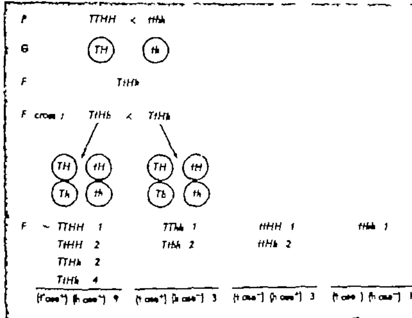


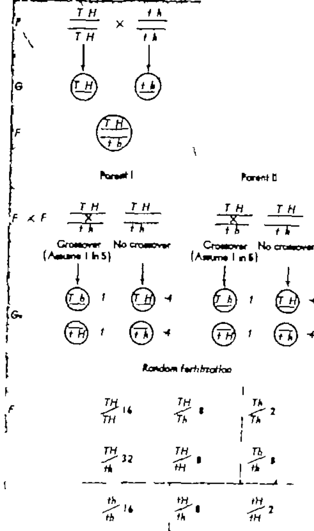
Fig. 33. The inheritance of two genes on separate chromosomes.

Linkage

For our third example, we will analyze the transmission of two genes linked together on the same chromosome. Chromosome assortment during meiosis would *by itself* not result in the separation (segregation) of linked genes. In the absence of crossover all the progeny would be phenotypically and genotypically like their parents. Assume that the tryptophan and histidine genes are linked on the same chromosome. A cross of the homozygous dominant $(T,H/T,H)$ by the homozygous recessive $(t,h/t,h)$ will give heterozygous F progeny $(T,H/t,h)$ (Fig. 34) Meiosis in the heterozygous progeny will normally result in the formation of only two types of gametes, (T,H) and (t,h) Thus, if two heterozygous F_1 progeny are crossed, we will find in the F only strains that are $(T,H/t,h)$ $(T,H/T,H)$ and $(t,h/t,h)$ all phenotypically like one or the other parent. No segregation of the parental genes on the chromosome has occurred.

However as we discussed in Chapter 3, during meiosis the intimate pairing between homologous chromosomes permits the occurrence of segmental exchanges, the phenomenon of crossing over The consequences of crossing over at an assumed frequency of one in five, are given in full in Fig 34.

By examining the figure, you will notice that all the F progeny are dominant for both genes, as is true of the F progeny that arise when the genes are in separate chromosomes. The difference, however is that both



F_2 Phenotypes: t ase hat — 64 t' ase hat — 10

t ase h'at — 16 t' ase h'at — 10

Parental phenotypic classes

Recombinant phenotypic classes (Note equal reciprocal recombination)

Fig. 34. The Inheritance of two linked genes

dominants are still on *one* chromosome. In the F cross, recombination of the dominant and recessive genes takes place through crossing over (Have you noticed that crossing over in the parental chromosomes has no genetic effect?) The types of gametes formed, their frequencies, and the full genotypic and phenotypic consequences of random fertilization are recorded in Fig. 34. Note that nine different genotypes are produced in the F_2 by the breakage-fusion recombination. Genes even when linked,

do segregate. Thus the final phenotypic ratio with 20 per cent (1 in 5) crossing over is 64 10·10 16 far different from the 9 3 3 1 ratio expected for unlinked genes. Study the figure carefully and devote some thought to it. Set up the following problem

$$\left(\frac{T\ H}{t\ h}\right) \times \left(\frac{t\ h}{T\ H}\right)$$

Assume a 20 per cent crossover frequency and solve it by writing every resulting genotype, phenotype, and the expected ratios. (For our illustration, a crossover frequency of 20 per cent was assumed, but remember *that the frequency is a function of the distance between the genes on the* chromosome.) You will be in a position to understand some of the fascinating work that has been done, and *is still being done, in the genetics* of higher organisms, in breeding and selection, in insect population control, and in the effects of radiation.

Sex Determination

Before we go on to a study of human genetics, we must discuss two additional subjects the genetic determination of sex and a curious event, chromosomal nondisjunction, first observed in studies on sex determination. In microorganisms, sexual reproduction arises from a mating-type system. In general, there are no important morphological differences between the mating types They are characterized by the fact that strains within a mating type are mutually infertile Strains from different mating types will crossbreed to form a partial or complete zygote. This compatibility difference is inherited as a single-gene difference. In true sexuality clear *functional and structural differences exist. There are two* gametic cells of different function, the egg cell and the sperm cell, with *elaborate functional* differences between the adults of the two sexes. In humans, many developmental differences are known to exist between the sexes, and these differences appear to reflect the action of many different genes, i.e., sex determination is multigenic. This is borne out by the fact that sex determination involves a chromosomal difference with its full complement of genes.

The chromosomal basis of sex determination can be seen clearly in the fruit fly Drosophila. Cells of the female have four homologous pairs of chromosomes, while cells of the male contain three homologous pairs (autosomes) and one unlike pair The unlike pair consists of one chromosome that is homologous to one in the female cells and another that is distinctly *dissimilar and found only in male cells. The chromosome found* in both cells is called the X chromosome, and the unlike chromosome *characteristic of male cells* is called the Y chromosome Females can transmit only X chromosomes, while males transmit either X or Y and in equal

frequency Clearly then, there is an equal probability that a newly fertilized zygote will contain either two X chromosomes, a new female, or one X and one Y a new male, thus providing for equality in numbers of both sexes.

The chromosomal basis of sex determination in man is superficially similar to that in Drosophila. The female has two X chromosomes in her cells, the male an X and Y This chromosomal distribution, however is not universal. In some organisms—the domestic fowl and some moths—the male has the equivalent of two X's and the female the X and Y In still others, the female normally has two sex chromosomes and the male only one—there is no "Y" chromosome and there are other variations.

The role played by sex chromosomes was clarified by a study of nondisjunction by Calvin Bridges. He found that on rare occasions, during meiosis, the X chromosomes paired, as expected, but then *did not separate* did not disjoin. They both went into one of the succeeding daughter cells. The other cell did not receive an X. All the other chromosomes were well behaved. These eggs, XX and O instead of the normal X, were then fertilized by either X or Y from the male to give XXX, XXY OX, and OY In Drosophila, XXX developed into an infertile female (called a superfemale—unfortunately a most inappropriate term) XXY was a fertile female, OX a sterile male, while OY was lethal, the egg did not hatch.

It is now known that nondisjunction can occur in many organisms, including man. Of particular interest is the fact that it apparently can involve any chromosome. The revelations provided by studies of nondisjunction in man in terms of both sex determination and the development of physical and mental abnormalities will be discussed in the next chapter

Genes and Man

History tells us that not very long ago man thought of his remote planet as the center of all things with the rest of the universe revolving respectfully around it. Scientific inquiry has demolished this small conceit but not man's ego. It is no wonder that he has made human genetics a subject of special interest. And it is in this part of genetics that he needs ingenuity for in obedience to cultural requirements, he must deny himself the free *use of a valuable experimental tool,* controlled mating.

Direct study of the genetic basis of human traits has depended largely on refined statistical analysis of geneological lines and the compilation of vital statistics. These methods have been forced upon us by the long reproductive cycle in man, about *thirty years per generation,* and, in the genetic sense, by unrestricted matings. Fortunately all the accumulated evidence confirms the proposition that the transmission and function of human genes correspond to those of other biparental diploid organisms. Let us begin with an examination of some very recent data derived from the introduction of new and promising methods.

The Chromosomal Basis of Gene Transmission

Surprisingly the number of chromosomes characteristic of the human species has long been a subject of controversy. Identification is difficult because many of the chromosomes are cytologi-

cally alike while others are small and difficult to detect. Recently human cells have been cultured in the laboratory by techniques (tissue culture) similar to those used with microorganisms, and, under these conditions, they provide excellent material for cytological investigation. By special methods, cell division can be arrested and the chromosomes maintained at the most favorable point for observation—on the equatorial plane just before separation. These studies reveal a basic chromosome number in man of 46, consisting of 23 pairs. Cells derived from females show 23 homologous pairs, while cells from males show 22 pairs and a twenty-third pair consisting of an X-chromosome and a shortened Y (Fig. 35). An immediate result of the development of tissue culture methods was the discovery in man of nondisjunction.

Some Congenital Diseases and Nondisjunction

It has recently been found that Mongolism, a serious neurological disorder accompanied by a characteristic Mongoloid appearance and by mental retardation, is associated in many cases with a chromosome count of 47. The rare child with 47 chromosomes is generally born to parents with normal counts of 46. The most reasonable explanation for this aberration is nondisjunction. An egg cell in which one of the chromosome pairs does not separate during meiosis would, upon fertilization, form a zygote with 47 chromosomes, 22 normal pairs and 1 triplet. In Mongolism, the triplet normally occurs in chromosome No. 21, and, for some unexplained reason, the additional chromosome alters development and results in the described neurological disorders.

Nondisjunction of other chromosomes, including the sex chromosomes, has been found and is associated with other disorders. Individuals are known whose cells carry two X-chromosomes and one Y. They suffer from a neurological defect known as Kleinfelder's Syndrome and show physiological and psychological abnormalities relating to sex determination and expression.

Nondisjunction of the sex chromosomes implies an interesting consequence. If a pair of chromosomes does not separate during meiosis, then one of the products of meiosis gets both chromosomes and another should get none of the chromosomes. When fertilized, the latter would have a chromosome number of 45 and be haploid for the missing chromosome. This has been verified. Individuals have been found whose cells contain an X but no Y. Phenotypically they are undeveloped females with no ovarian tissues (Turner's Syndrome). In addition to providing evidence for nondisjunction, the discovery of XO offers some interesting revelations into sex determination in man.

In Table 1 are listed some known genotypes relating to the sex chromosomes and the corresponding phenotypic expression in both man and Drosophila.

Fig. 31. (Right) A karyotype of human male cell, showing the 22 pairs of chromosomes. (Below) Photomicrograph of the chromosomes of human male cell. (Both courtesy Dr J H Tjio.)

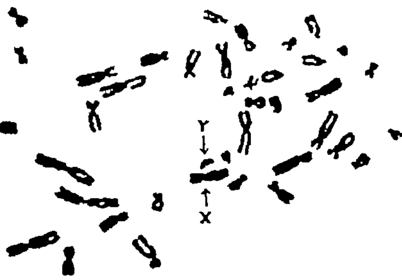


Table 1
The Phenotypic Expression of Various Genotypes in Man and Drosophila

Genotype ⟶	X	XX	XY	XXY	XXXXY
Human	UF	F	M	M	M
Fruit Fly	M	F	M	F	

F = Female M = Male UF = Undeveloped Female

Notice that in man, X and XX are female, whereas the possession of only one Y is sufficient to confer the male phenotype. In Drosophila, one X, with or without Y is male, while XXY is female. From this and additional evidence, it appears that Y in Drosophila carries few if any genes and none required for male development. In humans, the genes carried on Y appear to exert a powerful influence in sex determination, as is attested by a male with the bizarre chromosomal constitution, 5X,Y In general, it can be concluded that the possession of an extra chromosome or the omission of one of the chromosomes leads to developmental abnormalities There is no explanation for this but there is some interesting speculation which we defer to Chapter 9

Our discussion has touched on but a limited part of the evidence available from intensive researches now under way into the chromosomal basis of human heredity In the future, many more instances of the association of a specific disease pattern with chromosomal imbalance will probably be found. We know *no treatment for this type of disease.* Perhaps one day it will be possible to prevent nondisjunction or to rectify the physiological consequences of nondisjunction once it has occurred These are not idle hopes. Other human disorders with a different genetic basis are known. By making use of the facts and principles discussed in the preceding chapters, we have been *able to establish a rational course* of therapy in some of these cases As an illustration, we will discuss a disease called phenylketonuria.

Phenylketonuria

In the 1930's, it was observed that unusual amounts of a compound, phenylpyruvic acid, were excreted in the urine of certain patients afflicted with severe mental disorders, usually idiocy or imbecility The disease was called phenylketonuria and appeared to affect members of the same family A more detailed analysis of the pattern of family distribution turned up a result unexpected at that time. The inheritance of phenylketonuria could be explained on the basis of a single-gene difference. It was found that phenylketonuria occurred in brothers and sisters of affected persons, but rarely in their parents or in more distant relatives such as uncles, aunts, and cousins. The disorder was unusually frequent in consanguine-

ous marriages, i.e., those between cousin-cousin, uncle-niece, aunt-nephew (You realize that consanguineous mates have a common ancestor) Furthermore, even in affected families, phenylketonurics were in the minority

The observations all pointed to the association of this disease with a recessive allele of a single normal gene. Three genotypes could then exist in the population, the homozygous dominant with a normal phenotype, the heterozygote, also normal, and the homozygous recessive, the abnormal phenotype. The homozygous recessive condition accounts for the increased frequency of the disease in consanguineous marriages, since the probability is increased of a mating between two heterozygous individuals to give the required homozygous recessive. Once the genetics of the transmission of the disease had been solved, the rest became a matter of biochemical detection.

The biochemical basis of phenylketonuria lies in the metabolism of the amino acid, phenylalanine (Fig. 36) Part of the phenylalanine we ingest in our diets is normally oxidized to another amino acid, tyrosine. If, for some reason, the oxidation is blocked, phenylalanine is alternatively oxidized to phenylpyruvic acid, which is then excreted. Combine the genetic evidence (a single gene) with the loss of a single reaction and our suspicion falls, correctly on an enzyme. The recessive allele cannot direct the formation of the enzyme that catalyzes the conversion of phenylalanine to tyrosine. But how can this single loss lead to mental impairment?

Human beings are unable to synthesize many amino acids essential for protein formation—they must obtain them in their diet. Phenylalanine is one of these required amino acids, but tyrosine is not, since a sufficient amount of tyrosine is ordinarily formed by the oxidation of dietary phenylalanine. The normal diet, however provides both phenylalanine and an

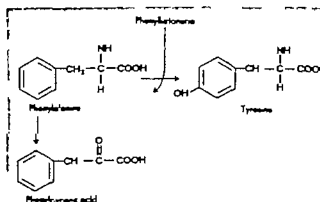


Fig. 14. The biochemical basis of phenylketonuria.

adequate amount of tyrosine, and thus the genetic loss of ability to convert phenylalanine to tyrosine does not impair growth. By itself the loss of the enzyme should be harmless. However since phenylalanine cannot be converted to tyrosine, an alternative mechanism takes over and causes the production of abnormal amounts of phenylpyruvic acid. The accumulation of phenylpyruvic acid, a secondary consequence of enzyme loss, appears to be responsible for the neurological damage.

The course of therapy evolved for this disease makes use of all the information outlined above If this disorder involves just the one enzymatic defect, it should be possible to rear such an individual without attendant mental impairment by maintaining dietary levels of phenylalanine just sufficient for protein synthesis but low enough to keep phenylpyruvic acid at a minimal and, hopefully nontoxic level.

This treatment has only recently been applied, and the data gathered so far are encouraging. Phenylketonuric children reared from birth on a carefully prepared diet containing subsistance levels of phenylalanine appear to grow normally both physically and neurologically Observations on their progress are still continuing, and there is reasonable hope that the disease may have been circumvented.

Phenylketonuria offers a valuable lesson when we are considering the effects of gene mutation. A genetic change that affects a specific biochemical reaction may indirectly bring about more extensive biochemical changes than the loss or inhibition of one reaction. If a cell loses its normal ability to carry out a reaction, say $A \longrightarrow B$ compound A may accumulate or may be metabolized to other compounds. Either event can have a profound metabolic effect and must be taken into account when assessing the over-all phenotypic effect of a single-gene mutation. Phenylketonuria also makes it clear that if a human disorder can be shown to correspond to a single gene difference, there is a strong probability that the difference between a normal and an abnormal individual is basically a difference in a single enzyme If we can recognize the reaction, we may be able to bypass the genetic defect.

The inheritance of galactosemia is another case in point. Galactosemia, a disease first noted in children, is inherited as a single-gene difference and involves the loss of a single known enzyme. As in the case of phenylketonuria, we find that the loss of a single biochemical reaction indirectly leads to a number of associated abnormalities, including mental retardation. Knowledge of the biochemical basis of the defect again permits rational treatment. The interested student can readily find the details in a reference given at the end of the book.

From these examples, we can see that the basis of gene action in man, control of biochemical reactions through the mediation of enzyme formation, is the same as that in microorganisms and other subdivisions of the plant and animal kingdoms.

Sickle-cell Anemia

In the cases cited above of inherited disease in man, we drew on information gathered from other sources, notably microorganisms to clarify the nature of the disease and to prescribe treatment. In turn, a series of brilliant studies on another human disease, sickle-cell anemia, has significantly increased our knowledge of gene action in all organisms.

In 1949 Linus Pauling and his colleagues discovered that the formation of an abnormal hemoglobin appeared to be the biochemical basis for the inherited trait, sickle-cell anemia. As you know hemoglobin is the major protein of red blood cells. It combines with oxygen in the lungs and carries it to all parts of the body In general, a decrease in the amount of atmospheric oxygen has no effect on the red blood cells of normal individuals. However it was noted as early as 1910 that under these conditions, the red cells of some people become elongated and take on an odd sickle shape. Ordinarily this does not affect the health of the individual, but in some cases red cell sickling is associated with severe hemolytic anemia. A study of the family distribution of red cell sickling showed it to be inherited as a single recessive gene. It was also possible to distinguish heterozygous individuals from homozygous recessives, since heterozygotes show sickling but no symptoms while homozygous recessives show sickling and suffer from hemolytic anemia. A family pedigree of this disease is given in Fig. 37

Pauling and his collaborators isolated the hemoglobin from normal and sickled cells—the latter from homozygous recessives—and discovered that the two hemoglobins differed in the electric charge on the surface of the molecules (Fig. 38) The hemoglobin of the heterozygote was

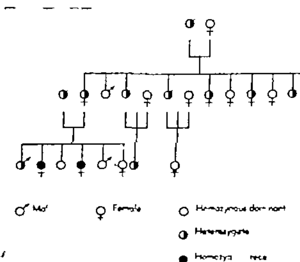
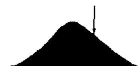


Fig. 37 Pedigree of sickle-cell disease (after Neel)



Normal



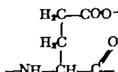
Sickle-cell trait



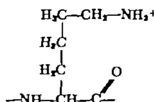
Sickle cell anemia

Fig. 38 Electrophoretic behavior of various human hemoglobins (after P. Ingram, et al.) Normal—homozygous dominant; sickle-cell trait—heterozygote; sickle-cell anemia—homozygous recessive. Arrow indicates reference point of origin.

then isolated and found to be composed of about equal amounts of both normal and abnormal hemoglobin (a nice demonstration that both alleles were functioning independently of each other). With the help of finger printing and amino acid sequence analysis as described in Chapter 4, Vernon Ingram showed that the two hemoglobins differed in one amino acid out of about five hundred. One glutamic acid residue in normal hemoglobin,



is replaced by lysine,



in precisely the same place in the protein to give sickle-cell hemoglobin. The amino acids are written with the charges they would have in the red cell, and explain the observed charge difference.

As far as can be determined at present, the substitution of glutamic acid by lysine is the only difference between the two hemoglobins, but this substitution has a profound effect on the physical characteristics of the formed protein. It results in the alteration of electrical charge as well as in a difference in biological activity, namely, the transport of oxygen. Very recent studies of other human hemoglobins have brought to light other single amino acid differences, suggesting that multiple allelism is as widespread in humans as in other organisms.

Multiple Alleles

The most striking case of multiple allelism in man occurs in the genes that determine his blood types. Blood characteristics differ from individual to individual, and one can observe this by a simple test. Mix a sample of red blood cells from one individual with serum from another (it would probably be advantageous at this point to reread the section on immunization in Chapter 4) A difference in blood type between the individuals will be revealed by the clumping of the red cells in the serum. The red cells will form large aggregates and settle to the bottom of the tube This phenomenon is called agglutination and indicates incompatibility between the bloods. Agglutination can be seen in the test tube, but will also occur in the blood stream; i.e. if blood is injected from one individual into a second and the blood is incompatible, agglutination of the red blood cells of the donor will occur Since agglutination can be fatal, careful tests must be made to determine what blood may be safely used in transfusion.

Agglutination requires two components a component in the serum and a component on the cells. The elements in the serum that are concerned with specific agglutinating activity are called antibodies and were described in Chapter 4. The specific properties of the cells that enable them to react with antibodies are antigens, and again these were discussed in the earlier chapter When the cells and the serum of two individuals, A and B, are mixed in all possible combinations, the cells of A, when put into the serum of A, give no agglutination. Cells of B added to the serum of B give no agglutination. But cells of B agglutinate in the serum of A, and, reciprocally cells of A agglutinate in B serum. All this can be explained by assuming that A individuals have an A antigen on their red cells and have antibodies to B in their serum. (They have no A antibodies in their serum, since that would be suicidal.) Similarly B individuals have B antigen on their red cells and antibodies to A in their serum. Transfusion of A to B or B to A, therefore, will give agglutination.

Blood types fall into four major groups with respect to the A, B antigens A, B AB and O As shown in Table 2, A carries the A antigen and B antibodies B carries the B antigen and the A antibodies AB carries both the A and B antigens on the same cell and no antibodies, while O

Table 2

Human Blood Groups

Genotypes	*Cell antigen*	*Serum antibodies*	*Blood group*
L L or $L^{A}I$	A	Anti-B	A
L L or $L^{B}I$	B	Anti-A	B
L L	A and B	None	AB
II	Neither A nor B	Anti-A and B	O

carries neither antigen and has both antibodies. The group to which an individual belongs is inherited as a single gene and the difference between an A and a B individual is an allelic difference.

The inheritance of blood type, then, corresponds to the inheritance of a gene that controls the formation of a specific antigen. The gene is called the L gene in honor of the great immunologist, Carl Landsteiner Individuals of type A carry an allele L^A which directs the formation of antigen A. Individuals of type B carry a second allele L^B which directs the formation of antigen B Individuals of type O carry a third allele, l, and neither A nor B antigen is synthesized. We would expect to find genotypes representing all possible combinations of the three alleles. These combinations consist of the six different genotypes listed in Table 2. The six genotypes give four phenotypes, A, B, AB, and O since L^A and L are dominant to l but show no dominance with respect to each other

The genetic consequences of this type of inheritance are clear An individual of blood group O might arise from the mating of two individuals of blood group A (i.e. from AO $\times$ AO) or from two individuals, one A and the other B (AO $\times$ BO) but an individual of blood group O could not have an AB parent. Conversely an AB individual could not possibly arise from a mating of two individuals of blood group O For this reason, blood grouping is of critical importance to legal medicine in helping solve such thorny cases as issues of disputed parentage. While the inheritance of the A and B antigens is reasonably well understood, the inquiring student may wonder how the L gene, while determining the formation of a specific antigen A, at the same time results in the formation of antibodies against B The best information is that the association of L and anti-B antibodies is accidental, but the problem is still open. It must be emphasized that other blood group systems in man are *not* associated with the simultaneous presence in the serum of antibody to the antigenic product of the alternate allele

Other blood traits in man are also known to be inherited as an allelic series. The Rh factor was discovered by testing human red cells with anti serum produced against red cells of the Rhesus monkey Some human bloods reacted with these antiserums, others did not, revealing that an antigen similar to one on the monkey cells was present in some human blood cells bu not in others. The antigen was called the Rhesus or Rh factor and it has proved of great interest since an association has been established between the Rh factor and hemolytic anemia of the newborn. Genetic investigations have revealed many different Rh alleles, and the inheritance of the Rh factor represents a multiple allelic series or a series of very closely linked genes While the Rh factor is an inherited trait, its relation to hemolytic anemia of the new born is an immunological problem.

In matings of an Rh^+ father and an Rh^- mother a situation can arise in which the Rh^- mother carries an Rh^+ embryo. You can readily figure out the genotypes of the two parents required for this situation. In

some cases, the Rh factor (antigen) of the embryo can cross the placenta and induce antibody formation in the mother (Since she is Rh$^-$ she will react to the Rh factor as she would to any other foreign antigen.) We thus have a situation in which the Rh factor of the embryo induces antibodies in the mother and these antibodies can now return and damage the embryo by agglutinating and destroying the embryo red cells (Fig. 39) Since the total amount of Rh factor in the embryo is small, the total amount of antibody formed in the mother will be limited and will have relatively little effect on the first born. With succeeding Rh$^+$ pregnancies and continued immunization, the level of anti-Rh antibodies will increase in the mother's serum, increasing amounts of antibodies can enter the fetal circulation, and this in time can give rise to a severe anemia in the fetus. After a few pregnancies, antibody levels can become dangerously high, and the newborn may require a complete transfusion to prevent death It is clear that knowledge of Rh compatibility in prospective parents is of vital importance. We can then be alert for the possible appearance of hemolytic anemia in the newborn child. If it does exist, a complete transfusion will save its life.

Still other antigenic differences are known for blood, and even in the ABO group new differences are still being uncovered In fact, human blood constitutes a magnificent laboratory for genetic experimentation. We have discussed the brilliant investigations of human hemoglobin and the inheritance of blood types and the Rh factor In the years ahead, it may well be possible that use can be made of circulating blood cells *in vivo* to study problems of cell populations in a manner similar to that in which we study bacterial genetics.

Up to now our discussion of genetics has been confined mainly to the transmission of single genes and their alleles and to the relationship of single genes to specific biochemical reactions. The inheritance of many characteristics, however appears to be governed by more than one gene.

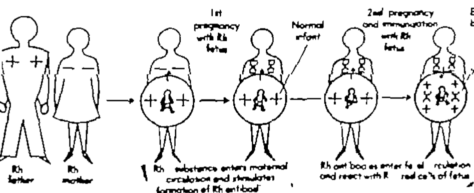


Fig. 39 Rh factor incompatibility (after Srb and Owen)

These include such traits as susceptibility to certain diseases, height, the development of the nervous system, skin color etc. An extended analysis of multigenic inheritance is beyond the intention of this book, but, because of its importance to human genetics, we will briefly describe one case

Skin Color

Let us see what would happen if two unlinked genes, each contributing equally were to determine the difference between black skin color and white. Let the capital letters Y and Y_2 stand for the black genes, and y_1, y_2 for the white alleles Assume white and black are homozygous for the two genes. A cross between black $Y Y_1 Y_2 Y_2$ and white $y_1 y_1 y_2 y_2$ would give the F_1, $Y_1 y_1 Y_2 y_2$, intermediate in color between the parents. A cross between F $Y_1 y$ $Y_2 y_2$, and white, $y_1 y$ $y_2 y_2$, would give three genotypes and three phenotypes $yy yy$ = white, $Y y$ $Y_2 y_2$ = the same as F $Yy yy$ = intermediate between F_1 and white. The three phenotypes would be in the proportion 1 1 2. (We have left out the subscripts in two genotypes since the *total number* of genes is the determining factor not whether it is an allele of the first or the second gene.) If you work out an $F \times F$ cross, you will find the results to be 1 white, 1 black, and 14 with varying amounts of intermediate pigmentation.

In human skin pigmentation, the genetic difference between yellow black, red, and white includes many genes Estimates vary for the total number involved, but the evidence points to at least eight and probably many more. Assume the number is ten, that they are unlinked, and the conditions stated above pertain. In a mating between two people both heterozygotic for every gene, 1 in about 1000 of their children would be either white or black, a rare occurrence the other children would be intermediate. On the other hand, a mating between white and intermediate could produce no children darker than either parent.

In this curtailed analysis of multigenic inheritance, certain simplifying assumptions were made Since a large number of genes govern the inheritance of skin color we would expect some of them to be linked and thus not able to make equal contributions. The conclusions we arrived at, however will not be badly distorted. This is affirmed by analysis of multigenic traits in plants and animals under conditions of controlled breeding.

In this chapter we have attempted to present an outline of the principles of human genetics. There are other aspects of human genetics, each with its unique contribution to the diversity of genetic mechanisms in man, that we cannot describe because of space limitations—for example, the transmission of color blindness and hemophilia, which follows a pattern of sex-linked" inheritance because the genes responsible for these traits are on the X-chromosome but not on the Y (Note that XX = female is normally diploid for a sex linked trait, but XY = male is haploid.) The significant thing is that the genetic material of man in its transmission

and action, is similar to that found in other organisms. We are thus increasingly sure that information obtained by study of microorganisms, insects, plants, and animals will be pertinent to the solution of the hereditary problems of man. Perhaps of greatest interest is our present conviction that human genetics itself will make important contributions to the basic problems of genetics, a statement that would have been considered implausible ten years ago.

Genes and Development

Genetic research during the past ten to fifteen years has been engrossed in the identification of the material basis of the gene, DNA, and of the mechanism whereby the gene functions in the living organism. In our description of some of the current research techniques, of the success that has been achieved, and the questions that arise with each new bit of information, a certain amount of bias inevitably has been introduced. We have implied, at least by emphasis, that nuclear inheritance is the sole mechanism operative in the transmission of heritable traits and that the gene functions by the determination of enzyme structure If this be gospel, then it must be reconciled with all the facts of biology Reconciliation is not easy

Consider but one example. Man starts from a fertilized egg cell, one cell which divides mitotically to produce two cells these divide mitotically to form four cells—and so on up to a few billion cells, all generated by mitosis, all with a gene content presumably identical with that of the first cell, the zygote. But we know that these billions of cells are not identical in form function and biochemistry: they are liver cells, nerve cells, blood cells, eye cells etc. Differentiation has occurred. We wonder how these differences are generated, how the cells take up their roles in such a precise and orderly fashion. We wonder in short, what it is that regulates and how regulation is carried out Is this an additional and exclusive property of the nucleus, or

is it an interaction between nucleus and cytoplasm, or cytoplasm and environment? These are problems basic to an understanding of development.

Acquired Traits

Heredity has long been thought to be uniquely associated with the nucleus. Mendel's laws, in effect, deny the inheritance of acquired characteristics except insofar as the genes themselves are directly affected. The role of environment in heredity has been a subject of speculation for many years. At the turn of the century a great deal of discussion took place about the relative roles of environment and cellular factors in heredity. People were aware of the subtlety with which living forms adapted to their environment: the exquisite camouflage of certain insects, birds, and arctic animals, the capacity of deep-sea animals to withstand crushing pressures, the enormous variety of habitats in which bacteria were found, in sulfur wells, in ocean bottoms, in the roots of certain plants, in hot springs. Biological history in general, indicated that better-adapted species evolved for a given environment. Did the environment play a direct role in this process by directing a specific change in the hereditary apparatus of the organism, or was this caused by random change followed by environmental selection?

This problem was put to experimentation by the German scientist, August Weismann, many years ago. The tails of an inbred strain of mice were removed at birth for a number of generations. Finally the mice were mated and their progeny left untouched. The tails of these offspring grew to normal length. This experiment is admittedly crude, but at the least it raised some doubts about the inheritance of environmentally induced traits, a strongly held belief at the time. More illuminating experiments have recently been done with the help of bacteria and antibiotics.

If you grow a large population of *E. coli* in the laboratory you will generally find that the population can be destroyed by streptomycin. By growing very large populations in the presence of streptomycin, you can obtain a population resistant to streptomycin. The progeny of these resistant bacteria are found to be resistant to streptomycin even when the antibiotic is removed from the growth medium. The question is, did streptomycin cause the resistance, or did it kill the sensitive bacteria and permit the growth of pre-existing resistant bacteria which arose by mutation independent of the presence of the antibiotic? An ingenious experiment by Joshua Lederberg provided a solution. He devised a method to show that in a large population of *sensitive* bacteria which had never been exposed to streptomycin, about one in ten million bacteria was resistant to streptomycin. He could pick out this bacterium and let it divide and form a very large population, and the entire population was *resistant* to streptomycin—all without the bacteria ever being exposed to the antibiotic.

This experiment, and many others, implies that living things, through gene mutation, are endowed with a certain potential for adapting to a new environment if it proves necessary But this potential is not infinite. Dinosaurs were displaced by other living forms, presumably because they could not meet the challenge of changing environmental conditions. The evidence today then, suggests that with random mutation, plus the endless reshufflings brought about by gene recombination, living forms can offer a wide variety of response to the environment. The concept that the environment can induce a specific heritable change is not supported by experimental data.

Russian geneticists, however have raised the problem anew Political philosophy in Russia demands that the environment play a hereditary role, for Marxist dogma decrees that social forces are capable of forging a fitter race. This doctrine is purported to have received experimental support from the work of Lysenko and his school, who have attempted to prove that permanent hereditary changes can be induced in plants by grafting, or in animals and birds by means of blood transfusion. Numerous attempts have been made in other countries to duplicate at least the major experiments on which their conclusions rest, but without success. At the present time, therefore we can only state that Lysenkoism has proven largely nonproductive in terms of genetic theory· it is essentially a revival of an outmoded genetic theory reapplied for political rather than scientific purposes.

Although experimental data do not support the theory that acquired traits are hereditarily transmitted, how do we classify such phenomena as bacterial transformation and transduction, for here we know that new genetic traits can be acquired by a microorganism from its surrounding medium? In these instances, however the traits have been acquired by the physical incorporation of genetic material, DNA, and thus the mechanism differs from the older concepts of how acquired traits are transmitted.

Although we may conclude that traits acquired from the environment may seldom become permanently transmissible traits, this does not answer the question about the uniqueness of the nucleus in the transmission of hereditary information. Are there elements in the cell for whose transmission the nucleus is insufficient?

Cytoplasmic Inheritance

There is a rather easy test for non-nuclear inheritance. In diploid genetics, we know that the egg contributes the bulk of the cytoplasm to the cell formed by gametic fusion. The sperm contributes relatively little. If there are elements in the cytoplasm that require the transmission of a cytoplasmic element rather than the nucleus, we would guess that such traits would be maternally transmitted. The progeny should then show only the maternal trait. Many instances of this type of inheritance are

known. A good example can be readily observed in plant variegation.

Variegated plants are those that have areas of pale green, white, or other colors in otherwise normally green leaves. The variegation can be symmetrical or it may be irregular. In Mirabilis, the plant commonly called the four-o'clock, the progeny of a cross between a pale and green plant is pale or green depending on the color of the maternal line—i.e., if the maternal line is pale, the progeny are pale; if the maternal line is green, the progeny are green. This is in contradiction to Mendelian genetics. Straightforward nuclear inheritance requires that the progeny of a cross between true-breeding pale and green plants be the same regardless of the maternal trait. The simplest way to account for this type of inheritance is to postulate that a non-nuclear element derived from the maternal plant determines this trait, and we find this to be correct.

The cytoplasmic elements concerned in variegation are the chloroplasts, the bodies carrying the photosynthetic green pigment, chlorophyll. The inheritance pattern of variegation suggests that the inheritance of a chloroplast depends on the transmission of a chloroplast itself. The data, in fact, indicate that if a plant were to lose its chloroplast, no nuclear element is present that can initiate its *de novo* formation. Chloroplasts, like genes, can undergo mutation and a mutant chloroplast gives rise to a mutant chloroplast, just as mutant genes reproduce mutant genes. This is why variegation is found. It is clear from a study of color mutants and variegation in plants that the cytoplasmic particulate element, the chloroplast, represents a cellular constituent that shows extranuclear inheritance and that the inheritance of a chloroplast requires at the minimum the transmission of a chloroplast itself. Inheritance of a nuclear gene is not sufficient.

We also know, however, that nuclear genes can affect the characteristics of chloroplasts. To explore the relationship of genes and semiautonomous cytoplasmic particles of this kind, we will discuss an example drawn from another organism. The general characteristics of cytoplasmic inheritance can be clearly demonstrated from the brilliant studies of Tracy Sonneborn and his collaborators. These investigators have studied the genetics of the single-celled protozoan, *Paramecium aurelia*. *Paramecium aurelia* is a small, ciliated organism with a normal mitotic vegetative cycle. Cytological studies reveal that a single animal contains three nuclei, one macronucleus and two diploid micronuclei. The macronucleus controls the cell during vegetative growth, while the micronuclei are concerned with meiotic reproduction. Sexual reproduction occurs by conjugation of two animals of opposite mating type (Fig. 40).

Conjugation initiates a series of nuclear changes that can be summarized as follows. When mating occurs, the macronucleus disintegrates and the two micronuclei each undergo meiosis, during which eight haploid nuclei are formed. Seven of these eight nuclei disintegrate, and the remaining haploid nucleus present in each mating animal undergoes one

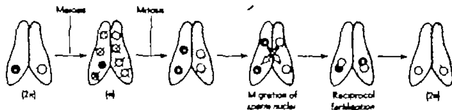


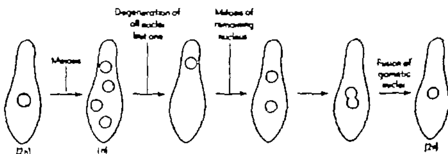
Fig. 40. Reciprocal fertilization P. ramecium (after Sonneborn)

mitotic division, giving rise to two identical haploid nuclei. One haploid nucleus from each animal is then exchanged, resulting in the formation of two animals, each carrying one haploid nucleus of its own and one contributed by its mating partner. These two haploid nuclei fuse to form a diploid nucleus which, in a series of developmental changes, gives rise to normal animals containing a macronucleus and two micronuclei. *Conjugation, therefore, results in the formation of two animals that are genetically* identical heterozygotes, but whose cytoplasms may differ.

Paramecium is capable of undergoing a second type of nuclear reorganization, one that does not require mating. Under appropriate nutritional circumstances Paramecium will undergo autogamy (Fig. 41). In this process, the macronucleus is again lost, and the two micronuclei each undergo meiotic division to form eight haploid nuclei. Again, as in mating, seven of the nuclei are lost. The remaining haploid nucleus undergoes a mitotic division. The two haploid nuclei then fuse to form the diploid nucleus which, in turn, undergoes a reorganization that reconstitutes a normal animal. The autogamy results in the formation of a genetically *homozygous* animal. These two different events, i.e., conjugation and autogamy, make Paramecium a remarkably interesting and useful genetic tool.

Because certain races of Paramecium can kill other races when present together in the same culture medium, certain strains are said to contain a "killer" trait. Strains of Paramecium, therefore, can be characterized as either "killer" or "sensitive" types depending on which is the assassin and which the victim. To determine the genetic basis of this killer trait,

Fig. 41 Autogamy in Paramecium (after Sonneborn)



we can cross a killer and a sensitive animal. After conjugation, the animals separate, and the phenotypes of the two cell lines derived from the two conjugants (exconjugants) can be determined. Since conjugation results in the formation of identical heterozygotes we would expect that both exconjugants should be either killers or sensitives, depending on dominance relationships Surprisingly however after conjugation the animals that were killers remain killers, while the animals that were sensitives remain sensitives.

The traits of the exconjugants, therefore, are determined by the parental cell and persist despite nuclear identity When animals are permitted to undergo autogamy and thus form homozygotes, revealing results are obtained. If the progeny derived from the sensitive exconjugants undergo autogamy only sensitive animals are recovered, but if the progeny of the heterozygous killer exconjugant undergo autogamy half the animals are sensitive and half are killers. A 1 1 segregation has taken place at autogamy clearly indicating that a genetic factor is segregated at autogamy

Inheritance of this trait shows certain odd features. Conjugation shows no nuclear transmission, but gene segregation is seen at autogamy In a study of this phenomenon, it was discovered that occasionally a killer and a sensitive enjoy prolonged conjugation and that under these circumstances, although the killer strain remains killer the sensitive is transformed into a killer Upon autogamy both cell lines show a 1 1 segregation for killers and sensitives Conjugation normally results in retention of the original trait, i.e., killers remain killers and sensitives remain sensitives. Prolonged conjugation, however permits sensitives to be transformed into killers. We know that the difference between normal and prolonged conjugation cannot be ascribed to a difference in nuclear exchange, but the difference could well reflect a difference in cytoplasmic exchange—the longer the conjugation, the more cytoplasm exchanged. It was postulated that some element in the cytoplasm of the killers could be transmitted to a sensitive animal during prolonged conjugation and cause the transformation of a sensitive to a killer This turned out to be true.

The killer trait requires that a specific element be present in the cytoplasm. If, during conjugation, this element is transmitted from killer to sensitive in the cytoplasm, transformation of sensitive to killer occurs. The fact that exconjugants show a segregation of killers and sensitives upon autogamy in turn suggests that in order for this cytoplasmic element to be functional, the animals must possess a specific nuclear gene. The cytoplasmic element is called "kappa, and the gene required for its repli
cation, *K*. A killer after autogamy is genetically *KK*, the sensitive *kk*. After mating, both sensitives and killers are heterozygous *K/k*. In the absence of the cytoplasmic transmission of kappa, which can occur only during prolonged conjugation, sensitives, although genetically capable of becom-
ing killers, remain sensitive, since ... ave no kappa, while the killers

with kappa present, remain killers. Upon autogamy half the animals are expected to become homozygous, *KK*, and half homozygous, *kk*, the latter group become sensitives.

These principles were derived in the absence of visual evidence of the presence of kappa. In subsequent experimentation, the predictions were confirmed. It has been shown that killer animals do possess a particulate element in their cytoplasm and that this element must be transmitted if the killer trait is to be established. It has further been confirmed that the animals must have a gene (*K*) in a specific configuration for this element to reproduce and act. In the absence of the transmission of the kappa particle, no nuclear element is present that can initiate the *de novo* formation of this particle. The kappa particle, in turn, requires for its duplication a specific genetic constitution of the animal. The killer trait, therefore, is gene-controlled but not gene-initiated.

The transmission of kappa in Paramecium reflects the general characteristics of cytoplasmic inheritance Many particulate elements of the cells show cytoplasmic inheritance, and in each case they show the general characteristics found in the inheritance of kappa, i.e., transmission requires transmission of the particle, but action and/or replication of the particle is controlled by the nuclear constitution of the cell. These traits may in general, be said to be gene-controlled but not gene-initiated. This mode of inheritance seems to be true for the inheritance of chloroplasts, and from the very fine studies of Boris Ephrussi and Piotr Slonimski, it appears that functional mitochondria are also inherited in this way In fact, we might suspect that a large number of the particulate elements of the cell require cytoplasmic transmission and that if they were lost, no nuclear elements would be present that could initiate their formation. The inheritance of cellular elements such as mitochondria, microsomes, etc., however is hard to study since cytoplasmic elements are present in profusion, and it is experimentally difficult to create situations in which no cytoplasmic transmission of a particle can take place In any event, *studies like those just described clearly indicate that the nucleus is not* the sole determinant of hereditary characteristics. Nuclear inheritance is of obvious importance, but the characteristics of a cell and of a whole organism require the transmission and interaction of many elements.

Cytoplasmic inheritance has been discussed primarily from the standpoint of the transmission of particulate elements. The cytoplasm, however may also play a decisive role in determining priorities for gene function In *Paramecium aurelia* as Tracy Sonneborn and Geoffrey Beale and their collaborators have shown, each animal is endowed with a number of genes, each of which controls the formation of a specific ciliar antigen. One antigen, however is predominantly formed. An animal, therefore, may be capable of forming antigens A, B C, D G but under specific conditions will form predominantly say B If we cross an animal forming B antigen with one forming G, the exconjugants and

their progeny will be genotypically identical. In spite of this, the progeny derived from the B exconjugant will form antigen B, whereas the progeny derived from the G will form G. Thus an antigen can be formed by an animal if and only if the proper gene is present. However, which of the antigens will be expressed will be determined by the cytoplasm, and, interestingly enough, by the interaction of the cytoplasm with the environment. For instance, drastic shifts in the environment, such as changes in temperature and in salt concentration, can cause the animal to synthesize a different antigen, but again only one for which the gene is present.

Thus the cytoplasm plays a decisive and intriguing role in determining the alternate and exclusive priorities in gene functioning. The nature of the cytoplasmic control is not understood, but, clearly, the cytoplasm is of importance in inheritance, and its role has many facets.

Regulation

A second problem mentioned at the beginning of this chapter concerns the action of all genetic material. Does all genetic material act alike and does it determine only the structural characteristics of product enzymes? There is no clear answer to these questions. We have known for many years that the expression of some traits is influenced by other genes (modifier genes). For instance, the intensity of pigment formation in plants can be modified so that some strains show more pigment formation than others. These intensity differences are genetically determined but involve many genes, each having a small effect. What do modifier genes do? They themselves may be genes that control the structure of specific enzymes and that secondarily, even accidentally, affect or modify the trait being measured, or they may be genes or genetic material that regulate the expression of other genes, perhaps turn them on and off, or change the rate at which they function, and thus differ in their action from genes that determine enzyme structure. At present, it seems that both kinds of effects may exist.

First, let us examine a possible mechanism by which a gene can modify the action of another gene through secondary effects. Many cells have an enzyme, beta-galactosidase, that is necessary for the utilization by the cell of lactose. (Beta-galactosidase catalyzes the conversion of lactose to glucose and galactose.) Mutation of this gene leads to loss of active enzyme and loss of ability to utilize lactose as an energy and carbon source. In order for a cell to take lactose up from the medium, however, the cell has to form a second enzyme. Mutation of the gene controlling the *formation* of this uptake enzyme (permease) leads to loss or lessened efficiency in taking up lactose. Mutation of either gene, independent events, leads to loss of ability to use lactose, but for different reasons. Thus mutation of the uptake gene can lead to modification of lactose utilization, and, if we were not a

would describe the uptake gene as a gene modifying the formation of beta-galactosidase.

Many genes are known to have such indirect effects. Thus it is abundantly clear that in an organized system, even if all the genetic material were to act by controlling the formation of specific enzymes, genes of quite diverse primary function can affect the activity of other genetically determined traits In an ordinary inheritance test, genes that exert a secondary effect on the trait under investigation appear experimentally as modifier genes, that is, as genes which alter the expression of the genetic trait under study

Another type of known genetic modification is exemplified by so-called suppressor genes, genes which suppress the phenotypic expression of other genes. Such genes need not be linked to the gene they affect, and their action is probably indirect. As an example, let us assume that the metal, zinc, inhibits the action of our favorite enzyme, t ase. A mutation resulting in excessive uptake of zinc would lead to suppression of t ase activity· thus the gene permitting zinc uptake would appear to act as a suppressor of the t ase gene. Like modifier genes, suppressor genes probably reflect biochemical interactions rather than diversity in the mechanism of gene action.

By now we have come sufficiently far in our description of gene action to entertain the astonishing hypothesis that all genetic material may not function by determining enzyme structure. Recent studies in bacteria suggest that some genes do not themselves act by specifying the structure of specific enzymes but regulate the action of other genes that do. These have been termed regulator genes as distinct from the familiar structural genes. Mutation of a regulator gene can lead to a marked change in the total amount of enzyme formed by the cell. A regulator gene has been found to regulate the formation of tryptophan synthetase in *Escherichia coli*, another gene regulates the formation of beta-galactosidase in the same organism. The regulator genes in these cases have an action which, in effect, turns a structural gene on or off The limited evidence available suggests that regulator genes may act by repressing enzyme formation. Enzyme repression is exerted via the cytoplasm, but the important fact is that it occurs in the absence of protein synthesis. Thus the mechanism, enzyme repression, is itself nonenzymatic, and the regulator gene controlling repression presumably does not act by itself to form a specific enzyme although it may form a specific repressing RNA.[1]

One regulator gene can coordinately repress the formation of several functionally related enzymes. For instance the gene which regulates t ase formation also regulates the formation of other enzymes required in the earlier steps of tryptophan biosynthesis. Regulator genes

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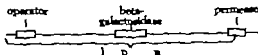
[1] For discussion of enzyme repression, see D. M. Bonner ed *Control Mechanisms in Cellular Processes* (New York Ronald, 1961)

which function by enzyme repression need not be linked to the genes whose action they regulate. They are effective even when located on another chromosome. Perhaps of greater interest are "operator" genes, genes which require close linkage for function, genes which may regulate directly at the gene level without an intervening cytoplasm. The concept of operator genes stems from the imaginative studies of the French microbiologist François Jacob, and his collaborators. To see how this takes place, designate an active structural gene as enz^{+}, its allele enz^{-}, the operator gene as op^{+} and its allele op. The two genes are closely linked. Two heterozygous diploid *combinations are possible*

$$\frac{enz^{-} \quad op}{enz \quad op^{-}} \qquad\qquad \frac{enz^{+} \quad op}{enz \quad op^{-}}$$

Only the second, cis configuration permits enzyme formation. The regulation by the operator gene, therefore, requires both linkage and cis configuration.

These facts and other data obtained by Jacob and his colleagues suggest that the operator gene can directly control the formation of the messenger RNA of the structural gene, and operator genes, like regulator genes appear to act by a mechanism which does not require the operator to form a specific enzyme. One operator gene may control the formation of several functionally related enzymes, provided the structural genes themselves are linked. For instance, the genes controlling the biosynthesis of histidine are linked and are all controlled by one operator. The genes controlling the formation of beta-galactosidase and the permease necessary for lactose uptake are linked in *E. coli* and both are regulated by the same operator gene. These observations have led the French workers to create a new genetic unit, "operon," which is a package deal consisting of an operator gene and the genes it regulates.



Continuing research is attempting to define the molecular basis of operator gene action.

These new data and new concepts imply that genetic material may be of two distinct types, one which prescribes the structure of formed enzymes and another which regulates the rate of enzyme formation. Whether the genetic material required for these differing kinds of gene action is similar or different remains a mystery. Regulator and operator genes, however, like structural genes, are carried in chromosomes, are capable of recombination, and thus are presumably of a DNA constitution.

In considering genetic effects, we must keep in mind that the quantitative expression of enzyme for

factors Enzyme formation occurs in the cytoplasm and requires the participation of an organized biochemical system. Any effect on one of the components of this system (all presumably under genetic control) obviously can affect the rate of enzyme formation both directly and indirectly In summary we should perhaps simply say that gene expression is never free of the genome and that a diversity of interaction clearly occurs, as can be seen from the names given to various components and their effects modifier genes, suppressor genes regulator and operator genes.

Genetic regulation is of particular interest to the field of growth and development. A single cell, with its entire complement of hereditary information, must interact with its environment in a prescribed sequence in time and space to produce the adult organism This is one of the most complicated events confronting the biologist today In this regard, let us turn for a moment to a consideration of the chromosomal trisomic conditions, Mongolism, Kleinfelder's syndrome, etc. described in Chapter 8. Through nondisjunction, an entire duplicate chromosome becomes incorporated into the nucleus and creates an abnormal phenotype Abnormal chromosomal constitutions are known in other living forms. In certain plants, normally diploid, duplication of the entire chromosome set occurs (polyploidy) The resulting 4N plants reproduce their 4N state and seem to get along perfectly well, whereas the addition of just one extra chromosome is troublesome. The difficulty appears to be one of imbalance. In the haploid, diploid, or polyploid state, in which full complements of chromosomes are present, gene products are apparently formed in varying amounts, but in a balanced condition. An additional chromosome seems to disorganize this situation This is another indication that genes do not act independently of one another in determining the phenotype of an organism, and that the balance of structure and regulatory genes may well be critical.

Among other problems, development poses the question of orderly genetic regulation, that is, what turns the action of a gene on and what turns it off? No satisfactory answer has been proposed. Perhaps development requires mutation, since mutation leads to functional differences, but if it does, the mutation must be a directed mutation, one that not only shows precision in terms of the genes concerned, but precision with respect to developmental time. In recent years, the magnificent work of Barbara McClintock and R. A. Brink with corn has revealed genetic elements that can induce gene mutation and direct the expression of specific genetic material. Even in this instance, however the precision required for differentiation is not present. The regulator genes and "operon" are also of interest to the problems of development even if they only furnish hints as to how regulation may be effected but they too lack the precision required by differentiation and development.

The cytoplasm has also to be reckoned with in development, for as we discussed in connection with antigenic variation in Paramecium, the

cytoplasm plays a role in determining the alternate and exclusive priorities in gene function. Many of the diverse phenomena just discussed may actually be closely related. From the study of bacteria, we know of cellular elements that enjoy both a chromosomal and cytoplasmic existence, for instance, lysogenic phage. Such elements have been termed episomes by the French workers, François Jacob and Élie Wollman. It may well prove true that the elements that can induce gene mutation and direct expression of specific genetic material in corn, the regulators of microorganisms, and the priority-determining capacity of the cytoplasm may finally find common ground in episomes.

Once again, we must emphasize that although the studies of the past ten years in the field of molecular genetics have been extraordinarily rewarding, the information obtained does not provide the solution for many problems in biology particularly those concerning differentiation and development. It may well be that genetic elements exist whose actions have not yet been described, we don t know At the minimum, development requires a full understanding of genetic regulation and of the interactions between nuclear and cytoplasmic elements. Genetics and development constitute a field of tremendous experimental possibilities, a field in which many new biological laws are likely to be discovered in the future.

Heredity and Hiroshima

In the course of the past sixty years, we have learned much about heredity and its underlying chemical basis. In turn, genetic knowledge has contributed to man's welfare on many levels. The material rewards to society derived from such knowledge have already been large, and will undoubtedly prove larger in the future. An exciting chapter in genetic history has been written in the production of high yield strains of hybrid corn, of high-yield wheat strains resistant to attack by the fungal parasites, rust and smut, which formerly caused nationwide crop failure, and in the more recent, slightly incredible, use of X-ray sterilized male blow flies to help eradicate the pestilent screw worm in the South. These all attest to the value of genetic knowledge in eliminating pestilence and in breeding animals, plants, and microorganisms to give hardier more productive strains and to increase the world's available resources of raw materials, antibiotics, and food.

Today a glance at the newspapers, with articles on population explosions, the drive of newly emerging nations for economic self-sufficiency and live polio vaccines obtained from mutant virus strains, will again remind you of some of the consequences of man's curiosity about the so-called ivory-tower concepts of the gene, DNA, and messenger RNA.

However let us turn our attention to still another topic of contemporary importance, caught up in recurrent ominous headlines, to a topic that now en-

gages the attention of people throughout the world—the global genetic problem arising from the harnessing and exploitation of atomic energy. We know that a portion of the stupendous energy released from nuclear fission and fusion is released in the form of high-energy ionizing radiations, and in the form of radioactive isotopes of elements such as strontium and carbon. Ionizing radiations are highly mutagenic. Before the advent of atomic energy the known mutagenic agents with which the human population had to contend were not of alarming concern. For example, ultarviolet light has low tissue-penetrating properties and though it causes burning of skin and may even induce skin cancer it has no marked mutagenic effect on other human cells. Chemical agents such as mustard gas are deleterious only when fools deliberately set them loose to be inhaled, and base analogues are ordinarily not present in the diet, although they may be used to treat or control various cancers. In general, these are mutagenic agents under our control. They can be used or forgotten at will.

Ionizing radiation, however is a different kettle of fish. High-energy radiation is capable of deep tissue penetration and is massively destructive and mutagenic. Since our environment is bombarded by a certain amount of background radiation from naturally occurring radioactive elements and from outer space, ionizing radiation is a mutagen we cannot entirely control and against which we are never completely protected. Our present concern is with the increasing amounts spewn into our environment from nuclear explosions. Each of us now receives more radiation than did our forefathers, and our children may receive more. Since the background radiation has shot upward in the past twenty years and since ionizing radiation is mutagenic, we must give thoughtful consideration to the possible genetic effects of this increase and of how great an exposure the human population can withstand.

Our concern with ionizing radiation is of two kinds, its effects on germinal tissue and succeeding generations, and its effect on somatic tissue, on us. Let us first consider somatic effects. Is there an association between somatic abnormalities, such as cancer and radiation? The frequency of leukemia, a cancer characterized by an excess formation of white blood cells can be increased by radiation. In the population of Hiroshima and Nagasaki that survived the atom bombs, there was a marked increase in leukemia during the subsequent five years. The association between leukemia and radiation is exhibited in other groups that have somehow been exposed to increased radiation. There is therefore a clear relationship between increasing amounts of radiation and increasing incidence of cancer

We know that radiation results in somatic mutation. In view of the correlation found between leukemia and radiation, does this mean cancer is mutational in origin? We do not know. We have expe evidence that some animal leukemias can be induced by a virus

we know that interactions between bacteria and bacterial viruses are modified by radiation. We cannot, therefore, conclude that the increase in leukemia associated with an increase in radiation arises as a consequence of mutation, for it could result from the interaction of irradiation, virus, and host. This is, perhaps, a fair assessment of the relation of radiation to cancer in general. Radiation could induce cancers for a number of different reasons. Since cancers have diverse origins, they may be caused by a virus, a mutation, or other factors. Regardless of the details, however there is an association between an increasing amount of radiation and an increasing incidence of cancer Radiation is clearly a potential somatic hazard.

We know of still other somatic effects of radiation, for a relationship between irradiation and aging has been observed. With increasing amounts of radiation, longevity is shortened. In fact, from animal experiments it appears that the shortening of human life may well be measured in days per unit dose. Again, increasing radiation must be viewed as a somatic hazard.

Uncontrolled release of nuclear energy also imperils the human population because it increases the abundance of certain radioactive elements For instance, radioactive strontium 90 formed as a consequence of hydrogen-bomb explosions is extremely dangerous, since it has a long half life and can replace calcium in bone. It is carried into the ionosphere and is ultimately washed down and widely deposited over the earth's surface by rain and snow From the soil, strontium 90 is absorbed by growing plants, the plants are ingested by cattle, and strontium 90 appears in milk and milk products. When taken into the body this radioactive isotope can take the place of calcium in the bone tissue, particularly in growing children. Strontium 90 in bone constitutes small centers of radioactivity and, if present in sufficient amounts, can give rise to bone cancer and a variety of other abnormalities. This is truly a new cycle of nature, a "twentieth-century cycle. The amount of strontium 90 has already been markedly increased by nuclear explosions, and we have no clear-cut answers as to how much strontium 90 can be safely tolerated in the human diet or how big an increase in the total amount of strontium 90 can be absorbed without adverse effect on the population as a whole.

Safety levels in man are difficult to determine They require long term study but our restless world denies us time. Decisions affecting the use of nuclear energy must be made, but, unfortunately our information about the deleterious effect in man per unit increase of radiation is inadequate to permit us to offer unequivocal biological advice. The thoughtful person must agree that an increase in radiation is attendent with hazard, and that any political decision leading to an increase in radiation must be made on the basis of the gravest, most soul-searching considerations

A second problem posed by bomb testing is the effect of ionizing

radiation on germinal tissue. There is no question that mutation rates are enhanced by radiation. This was shown years ago by H. J. Muller with X-rays and Drosophila. By far the great majority of such mutations are lethal or deleterious. As background radiation increases, the average dose received by each of us increases, and presumably the total number of mutations present in our gametic cells also increases. If the total number of transmissible mutant genes is on the increase, the number of genes having a deleterious developmental effect obviously must also rise. For the sake of future generations, we desperately need information on how big an increase in the genetic burden a human population can withstand and survive!

In the populations of the cities of Hiroshima and Nagasaki that survived the atom bomb, despite the observed increase in frequency of leukemia, *no substantial* rise in the frequency of spontaneous abortions or in the number of stillborn infants has been noted. These findings, though fragmentary, are of interest, since the rate of spontaneous abortions and stillbirths perhaps should provide some clue about the increase in dominant mutant lethal genes in the irradiated population, and the increase in dominant lethals in turn might give at least a rough estimate of the increase in recessive lethal genes. But no increase was found. Perhaps dominant lethals in humans are rare compared to recessive lethals, or perhaps dominant lethals are quickly filtered out by cell death. Inbreeding experiments are necessary to answer this point, but such experiments are denied us.

Many indirect methods have been used to estimate the so-called "genetic load" (number of recessive lethal and deleterious genes carried in the human population). Analysis of the vital statistics of various small populations in which consanguineous marriages have occurred for many generations permits a rough estimate of genetic load. The estimates have varied, but in general they have indicated smaller load than would have been anticipated from studies of other organisms. For example, relatively large numbers of recessive lethals can accumulate and be maintained in laboratory populations of Drosophila, with a consequent reduction in the vitality of the population when inbred.

In human populations, the number of recessive lethals appears to be relatively small. It must be emphasized that the data on human populations are few and the analysis is uncertain. However, it is possible that lethals do not accumulate in man, but are eliminated within the first few divisions of the fertilized egg. If this is true and if the data obtained from the Nagasaki and Hiroshima populations illustrate what we can expect in human populations, then the evidence suggests that an increase in radiation may constitute a greater somatic than a germinal hazard, but irradiation and its effect on the genetic load of the human population remain virtual unknowns.

One fact does remain crystal clear. Increased radiation represents a

serious human hazard, a hazard of recent origin and one that must be studied in detail. In the absence of overriding political considerations, background radiation obviously should not be increased without a sounder knowledge of the biological consequences of such an increase. How can more detailed information about the effects of radiation on human populations be obtained? The answer in part, must come from population studies of other organisms, and it also hinges on how successful we are in securing detailed information about the genetics of man. As mentioned earlier techniques are now being developed that in time may enable us to grow differentiated cells in tissue culture, and we may soon be able to study differentiated human cells much as we now grow and study bacterial cells. Perhaps the methods that have proven fruitful in defining genetic mechanisms in microorganisms will then prove fruitful in defining genetic problems in man, including the effects of radiation. The problems are immediate and urgent and require the thoughtful attention of all of us.

All of heredity cannot be presented in this slim volume. We have attempted to present the current ideas on the chemical basis of heredity and its mechanisms of action in the living cell. In addition, we have described some of the latest trends in genetic research, the problems that confront us, and the expectations that stimulate us. To do this, we have touched but lightly on the contributions of the giants of the past. And we have omitted, or merely hinted at, many other fields of genetic inquiry that are bustling and productive. If we have succeeded in whetting your appetite, we leave it to your curiosity to explore more deeply into the science of genetics. In our own biased opinion, we think that the future of genetics can be simply stated by these lines from a poem by e.e. cummings

—listen there's a hell
of a good universe next door: let's go

Selected Readings

There are many excellent texts of general genetics. We refer to but two of these and one collection of epic papers.

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